

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
 - TEXT CUT OFF AT TOP, BOTTOM OR SIDES
 - FADED TEXT
 - ILLEGIBLE TEXT
 - SKEWED/SLANTED IMAGES
 - COLORED PHOTOS
 - BLACK OR VERY BLACK AND WHITE DARK PHOTOS
 - GRAY SCALE DOCUMENTS
-

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problems Mailbox.**



Review

Size matters: use of YACs, BACs and PACs in transgenic animals

Patricia Giraldo & Lluís Montoliu*

Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Campus de Cantoblanco, 28049 Madrid, Spain

Received 15 August 2000; revised 20 October 2000; accepted 26 October 2000

Key words: animal transgenesis, bacterial artificial chromosome, BAC, functional genomics, P1-clone, P1-derived artificial chromosome, PAC, yeast artificial chromosome, YAC

Abstract

In 1993, several groups, working independently, reported the successful generation of transgenic mice with yeast artificial chromosomes (YACs) using standard techniques. The transfer of these large fragments of cloned genomic DNA correlated with optimal expression levels of the transgenes, irrespective of their location in the host genome. Thereafter, other groups confirmed the advantages of YAC transgenesis and position-independent and copy number-dependent transgene expression were demonstrated in most cases. The transfer of YACs to the germ line of mice has become popular in many transgenic facilities to guarantee faithful expression of transgenes. This technique was rapidly exported to livestock and soon transgenic rabbits, pigs and other mammals were produced with YACs. Transgenic animals were also produced with bacterial or P1-derived artificial chromosomes (BACs/PACs) with similar success. The use of YACs, BACs and PACs in transgenesis has allowed the discovery of new genes by complementation of mutations, the identification of key regulatory sequences within genomic loci that are crucial for the proper expression of genes and the design of improved animal models of human genetic diseases. Transgenesis with artificial chromosomes has proven useful in a variety of biological, medical and biotechnological applications and is considered a major breakthrough in the generation of transgenic animals. In this report, we will review the recent history of YAC/BAC/PAC-transgenic animals indicating their benefits and the potential problems associated with them. In this new era of genomics, the generation and analysis of transgenic animals carrying artificial chromosome-type transgenes will be fundamental to functionally identify and understand the role of new genes, included within large pieces of genomes, by direct complementation of mutations or by observation of their phenotypic consequences.

Overcoming position effects in transgenic animals

The generation of transgenic animals is a routine method in many laboratories worldwide. Transgenesis is commonly applied to study gene function in development and disease, to devise new animal models of human genetic diseases or to produce recombinant proteins in fluids, mostly milk, of transgenic animals. However, there is still a major limitation in the method, namely, the uncertainty about the expression of each transgene. This is mainly caused by the

stochastic event of transgene integration within the host genome and the nature of the transgenic constructs. It is accepted that host sequences surrounding the place of transgene integration can modify the expected expression pattern, potentially causing it to be ectopic, weak or even undetectable. This is currently interpreted as the result of chromosomal position effects (Wilson et al., 1990; Sippel et al., 1997). In addition, the limited knowledge of regulatory sequences for most genes favour the use of partial, often uncharacterised, genomic fragments that frequently function poorly in gene transfer experiments (Palmiter & Brinster, 1986).

* Author for correspondence: E-mail: montoliu@cnb.uam.es

A number of strategies have been proposed to overcome these position effects and thus increase the probability of optimal expression for transgenes integrated at random locations. The incorporation of homologous intronic sequences in transgenic constructs were among the first suggestions recognised to improve expression of transgenes (Brinster et al., 1988; Whitelaw et al., 1991). Heterologous introns have also been routinely used in transgenic experiments and shown to improve expression in some cases (Choi et al., 1991; Palmiter et al., 1991). The inclusion of specific sequences such as scaffold/matrix-attachment regions (S/MARs) (McKnight et al., 1992; but see Gutierrez-Adan & Pintado, 2000), locus control regions (Grosfeld et al., 1987; Ganss et al. 1994; Montoliu et al. 1996; most recently reviewed in Li et al., 1999) and insulators (Chung et al., 1993; Taboit-Dameron et al., 1999) have been reported to ameliorate transgenic expression. The adjacent co-integration of an abundantly expressed transgene has also been shown to rescue cDNA-type constructs present as a second neighbour transgene (Clark, 1997).

At best, targeting the site of integration by homologous recombination in ES cells (i.e., knock-in) would virtually solve any chromosomal position effects since the transgenic construct would then be controlled by all regulatory sequences present in the chosen endogenous locus. Alternatively, particular locations in the host genome can be selected according to their capacity to allow adequate expression patterns of experimental transgenes (Wallace et al., 2000). This strategy has been recently applied in transgenic sheep obtained by nuclear transfer from cultured somatic cells and served to identify the ovine $\alpha 1(I)$ procollagen (*COL1A1*) locus as a permissive candidate for the insertion of therapeutically useful transgenes to be expressed in the milk at optimal levels (McCreath et al., 2000). Figure 1 summarises the above-mentioned strategies devised to overcome position effects in transgenic animals.

Position effects support the notion that genes are organised on chromosomes as contiguous but independent units referred to as expression domains (Elgin, 1990; Laemmli et al., 1992; Dillon & Grosfeld, 1994). These expression domains are believed to remain insulated from neighbouring sequences and thought to include all regulatory elements that are necessary for correct gene expression. Thus, it is not surprising that standard transgenic constructs lacking most or some of these crucial regulatory sequences might display position effects when integrated ran-

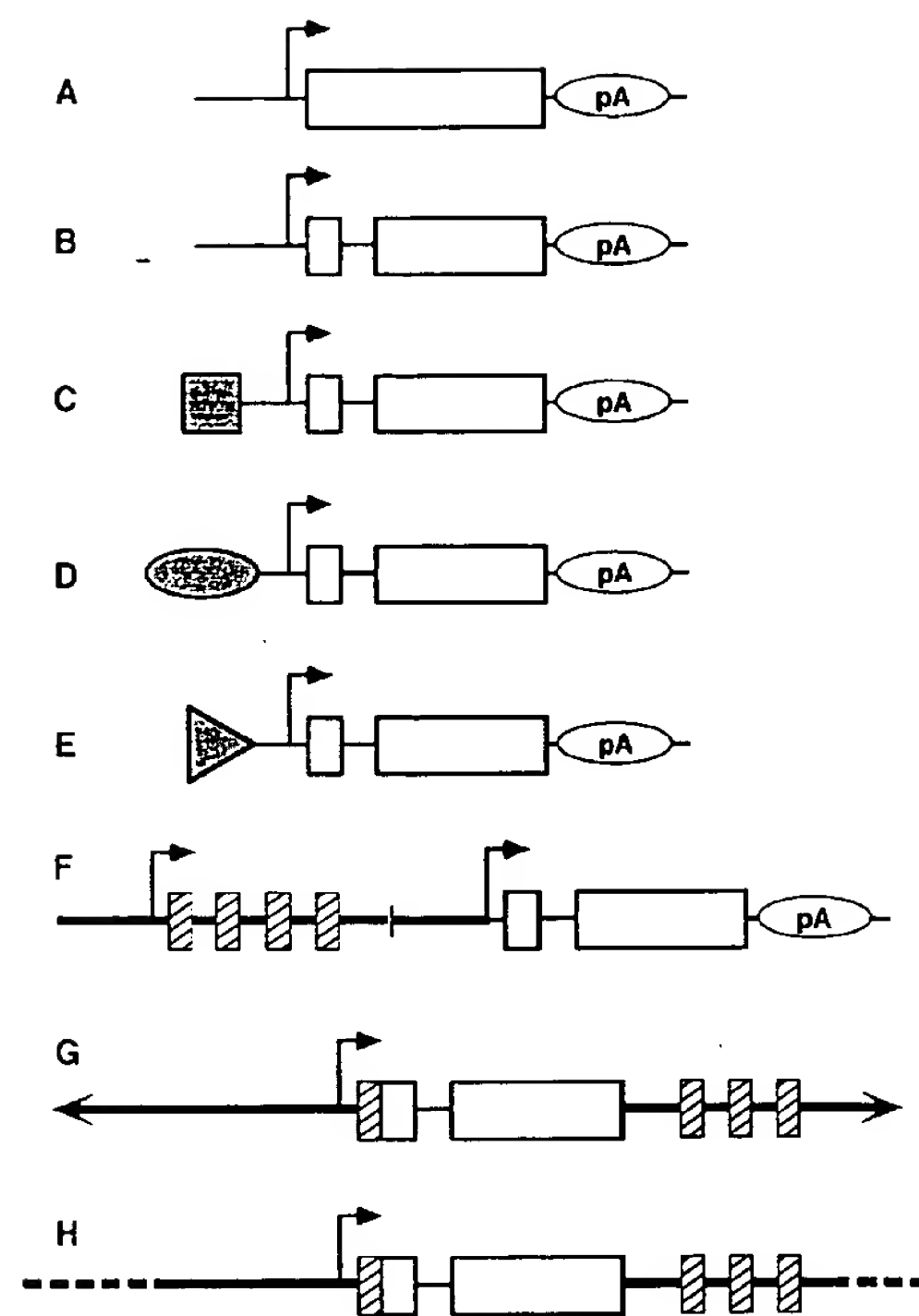


Figure 1. Diverse strategies devised to overcome position effects in transgenic animals (A) basic cDNA-type transgenic construct; (B) addition of intronic (homologous and heterologous) sequences; (C) addition of S/MAR sequences; (D) addition of a locus control region; (E) addition of insulating sequences; (F) transgene rescue by co-integration of an abundantly expressed transgene (stippled boxes); (G) use of genomic sequences contained within an artificial chromosome-type of transgenic constructs; and (H) gene targeting, knock-in. Symbols used: white rectangles (coding region of a gene), grey rectangle (S/MARs), grey oval (Locus Control Region), grey triangle (insulator), stippled box (coding region/exons of another gene, used to drive the expression of the transgene), white oval with pA (polyadenylation signal).

domly within the host genome. Most of the strategies devised to overcome such position effects have reported the progressive addition of regulatory elements, as a successful approach to improve the expression of transgenic constructs in a significant manner. Figure 2 shows a graphic representation of an expression domain along with the expected performance of different versions of a corresponding transgenic construct, progressively including more regulatory elements. Theoretically, the inclusion of all regulatory elements that are associated with a given expression domain in a transgenic construct would guarantee optimal expression levels in transgenic animals regardless of position of integration. Such conditions are present in artificial-

Figur
mally
ident
elem
gene
circle

chro
due
we
tran

tific
have
199
hart
Um
ders
to e
ent
gen
the
don
gen
with
cho
kno
Stra
et a
199
Pet

Tr?

YA
stal

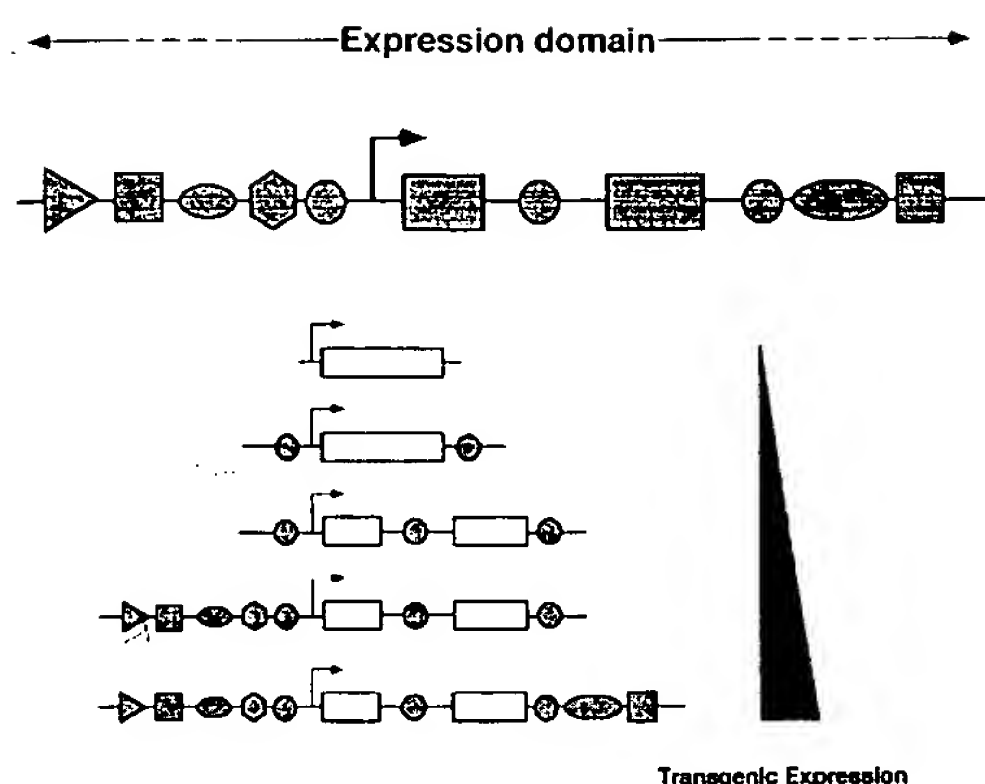


Figure 2. The expression performance of transgenic constructs normally depends on the presence of sufficient regulatory elements that identify an expression domain. The progressive addition of these elements usually correlates with an improved expression of transgenes. Symbols used are as in Figure 1 with the addition of: grey circles (enhancers), grey hexagon (repressor).



position effects
genic construct;
ous) sequences;
f a locus control
transgene rescue
nsgene (stippled
thin an artificial
) gene targeting,
egion of a gene),
ol Region), grey
xons of another
white oval with

the strategies
is have repor-
elements, as
expression of
iner. Figure 2
pression do-
e of different
onstruct, pro-
nents. Theor-
elements that
domain in a
timal expres-
ss of position
it in artificial-

chromosome type vectors (YACs, BACs and PACs), due to their large cloning capacity. In this review we will discuss the use of these vectors in animal transgenesis.

The benefits and applications of using yeast artificial chromosomes (YACs) for animal transgenesis have been previously reviewed (Montoliu et al., 1993, 1994; Forget, 1993; Jakobovits, 1994; Lamb & Gearhart, 1995; Peterson, 1997a; Peterson et al., 1997b; Umland et al., 1997; Huxley 1998; Camper & Saunders 2000). The use of YACs in transgenesis is likely to ensure position-independent, copy-number dependent and optimal levels of expression of the transgenes, provided all regulatory sequences needed for the establishment and maintenance of the expression domain are located within the YAC. Thus, YAC transgenes can circumvent most position effects observed with standard constructs, being the recommended choice when regulatory sequences of a gene are not known (Lamb et al., 1993; Schedl et al., 1993b; Strauss et al., 1993; Hodgson et al., 1996; Ainscough et al., 1997; Fujiwara et al., 1997; Hiemisch et al., 1997; Porcu et al., 1997; Zweigerdt et al., 1997; Peterson et al., 1998; Li et al., 2000).

Transgenic animals generated with YACs

YACs are eukaryotic cloning vectors capable of the stable maintenance of genomic fragments of DNA

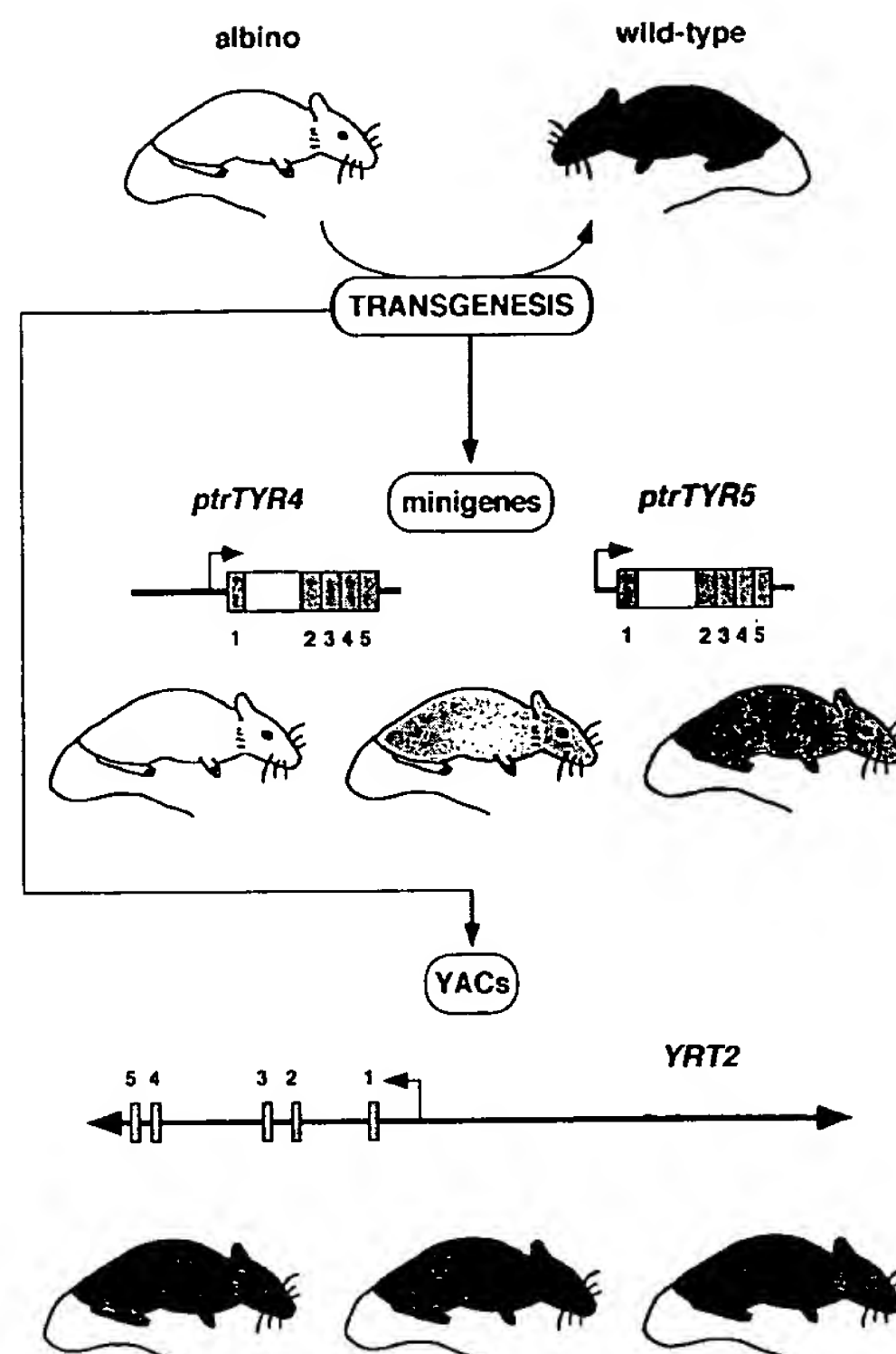
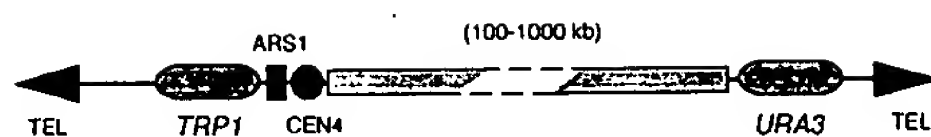


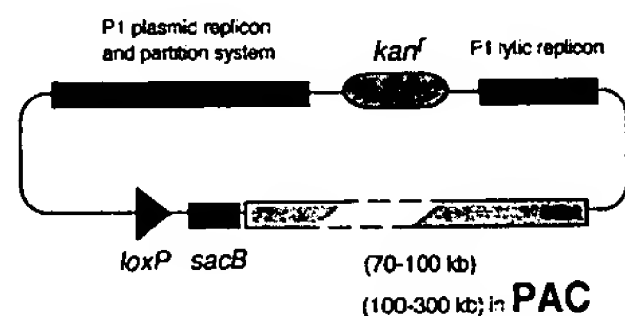
Figure 3. YACs versus standard constructs in transgenic mice, an example: rescue of the albino phenotype by introduction of functional tyrosinase constructs into mice. Standard minigene-type of constructs (ptrTyr4, 5.5 kb and ptrTyr5, 280 bp of tyrosinase promoter and upstream regulatory sequences, as reported in Beermann et al. (1990) and Klüppel et al. (1991), respectively) result in mice with variable expression of transgenes, showing pigmentation phenotypes weaker than that of wild-type animals. In contrast, YAC-type of constructs (i.e. YRT2, 250 kb encompassing the whole mouse tyrosinase locus, as reported in Schedl et al. (1993b)) produce transgenic mice indistinguishable of wild-type animals, without pigmentation variability and showing position-independent and copy number-dependent transgene expression.

larger than 1 Mb (Burke et al., 1987). YACs are linear DNA molecules and are generated from vectors (such as pYAC4, Kuhn & Ludwig, 1994) that provide all functional elements for their maintenance in yeast cells as artificial chromosomes (Figure 4, Green et al., 1999). Their vast cloning capacity, compared to standard cloning vectors (plasmids, phages and cosmids), made them very attractive for gene transfer experiments. Several groups tested this hypothesis and evaluated their suitability for transgenesis in mice. Thus, in 1993, a number of independent teams succeeded in generating the first transgenic mice with YACs.

YAC



P1 clone



BAC

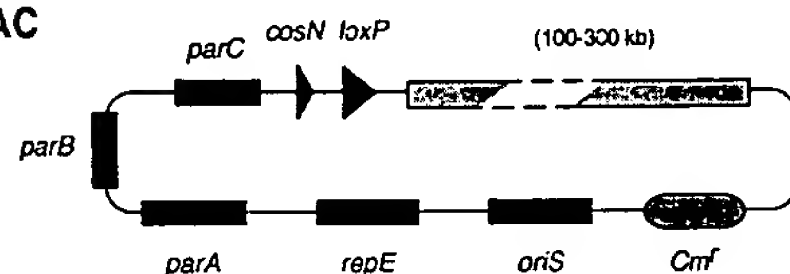


Figure 4. Schematic representation of a YAC, a P1-clone, a PAC and a BAC. Functional elements in each vector are shown in black. Selectable marker genes are shown in grey. Heterologous insert DNA is shown as a stippled discontinuous box. YAC, P1-clone, PAC and BAC modules shown here are derived from vector pYAC4 (Kuhn & Ludwig, 1994; Genbank U01086), pAd10SacBII (Pierce et al., 1992; Genbank U09128), pCYPAC2 (Ioannou et al., 1994, sequence derived from Genbank U09128, map available in <http://www.chori.org/bacpac/>) and pBe-loBAC11 (Research Genetics, Genbank U51113, map available in <http://www.tree.caltech.edu/>), respectively. P1-clones and PACs share vector sequences and only differ by their respective upper size limit on insert length, as indicated. Abbreviations: TEL, *Tetrahymena* telomere-derived sequence; CEN4, yeast *CEN4* centromere; ARS1, yeast autonomous replicating sequence 1; *TRP1*, yeast *TRP1* gene; *URA3*, yeast *URA3* gene; *loxP*, *loxP* site recognised by the Cre-recombinase protein; *kanf*, kanamycin-resistance gene; *sacB*, bacterial gene used in positive selection for cloned inserts; *cosN*, *cosN* site from bacteriophage λ which may be cleaved by bacteriophage λ terminase; *oriS*, *repE*, *parA*, *parB* and *parC*, genes derived from the F factor of *Escherichia coli* needed for the autonomous replication, copy-number control and partitioning of the BAC; *CmR*, chloramphenicol-resistance gene. Not drawn to scale.

Three groups reported their pioneer work in two journals within a week of one another (Jakobovits et al., 1993; Schedl et al., 1993b; Strauss et al., 1993). Remarkably, three different techniques were described to deliver YAC DNA to the germline of mice: pronuclear microinjection of gel-purified YAC DNA (Schedl et al., 1993b), lipofection of YAC DNA into ES cells (Strauss et al., 1993) and yeast spheroblast fusion with ES cells (Jakobovits et al., 1993). Two of these YAC transgenes, the mouse tyrosinase and the collagen (*COL1A1*) genes, demonstrated transgenic

expression at levels comparable to the corresponding endogenous genes (Schedl et al., 1993b; Strauss et al., 1993). Furthermore, analysis of several independent transgenic mouse lines carrying multiple copies of the YAC tyrosinase transgene permitted to prove position-independent and copy-number dependent expression (Schedl et al., 1993b). This study showed the faithful rescue of the albino phenotype of recipient animals by a 250 kb YAC tyrosinase transgene, as compared to the variability in pigmentation levels obtained with previous standard (and much smaller) tyrosinase constructs (Beermann et al., 1990; Tanaka et al., 1990; Kluppel et al., 1991). The comparison between standard (plasmid) and YAC tyrosinase transgenic mice is presented in Figure 3 and illustrates the generally good performance of YAC transgenes in gene transfer experiments observed in this particular and most other examples. Faithful expression was demonstrated with a 680 kb *Myf-5* YAC transgene (Zweigerdt et al., 1997), whereas previous attempts made with standard *Myf-5* transgenes driven by 5.5-kb 5'-upstream sequences failed to recapitulate the precise developmental expression pattern (Patapoutian et al., 1993). Similarly, a 130 kb YAC transgene containing both the *Igf2* and *H19* genes, was shown to display genomic imprinting effects according to their endogenous counterparts (Ainscough et al., 1997), in contrast with the previously described imprinting of mini-*H19* transgenes, which only occurred at multi-copy loci, inconsistently and prone to genetic background effects (Bartolomei et al., 1993; Pfeifer et al., 1996; Elson & Bartolomei, 1997). A number of independent experiments further confirmed the high performance of YAC constructs and their potential to overcome position effects in transgenic mice, according to the prediction of the model (Lamb et al., 1993; Strauss et al., 1993; Hodgson et al., 1996; Montoliu et al., 1996; Fujiwara et al., 1997; Hiemisch et al., 1997; Porcu et al., 1997; Peterson et al., 1998; Li et al., 2000). In most of these cases YAC transgene expression was found comparable to that of endogenous levels and largely determined by transgene copy-number. In addition, position-independent expression has been reported also in YAC constructs stably transfected in cells (i.e., Asselbergs et al., 1998; Vassilopoulos et al., 1999).

Some β -globin YAC transgenes, containing the β -globin LCR, have been reported to display uniform expression but position effect variegation in mice (Alami et al., 2000). However, these data are in good agreement with previous experiments carried out

in the
the
may
cific
con:
Thu
serv
resc
(Lal
the
requ
(Lal
lecu
ider
mul
der
hav
fer
of l
tran
Zot
tran
toir
unr
(Gr
et a

mar
bee
AP,
pro
and
fer
ani
pat
& c
lev
fea
anc
mic
nev
dis
et a

the
cor
dro
ge
cor
reg

corresponding Strauss et al., independent copies of the drove position-nt expression and the faithful pient animals as compared obtained with rosinase con- et al., 1990; etween stand-nsgenic mice the generally in gene trans-ular and most demonstrated veigerdt et al., le with stand-b 5'-upstream ecise develop-et al., 1993). ontaining both o display gen-ir endogenous), in contrast g of mini-H19 i-copy loci, in-ground effects 1996; Elson & endent experi-mance of YAC come position to the predic-Strauss et al., u et al., 1996; , 1997; Porcu et al., 2000). ne expression ogenous levels py-number. In on has been re-transfected in lopoulos et al.,

containing the o display uni-egation in mice se data are in ents carried out

in the endogenous mouse β -globin locus, suggesting the existence of unknown regulatory sequences that may compensate for LCR function when this specific sequence is removed from its endogenous normal context (Epner et al., 1998; Bender et al., 2000). Thus, the suboptimal performance occasionally observed with some YAC transgenes, such as the partial rescue of *GATA-3* mutant mice by YAC transgenes (Lakshmanan et al., 1998), is normally explained by the absence of additional regulatory elements that are required for correct expression pattern of the gene (Lakshmanan et al., 1999).

The use of YACs has been fundamental in molecular complementation of mutations, allowing the identification of new genes by transgene rescue of mutant phenotypes (i.e. Morgan et al., 1998; Majumder et al., 1998; Slee et al., 1999). Other applications have been explored with this new technique. The transfer of large genomic units enabled the production of human immunoglobulin light and heavy chains in transgenic mice (Davies et al., 1993; Choi et al., 1993; Zou et al., 1996) and, eventually, the generation of transgenic mice producing an almost complete repertoire of human antibodies in their sera from genomic unrearranged immunoglobulin loci cloned into YACs (Green et al., 1994; Fishwild et al., 1996; Mendez et al., 1997).

The overexpression of genes associated with human disease in mice via YAC transgenesis has also been investigated. For example, YACs containing the *APP* gene (>400 kb), encoding the amyloid precursor protein that accumulates abnormally in Alzheimer and other neurodegenerative diseases have been transferred to the germ line of mice aiming to produce animal models that were useful to study these human pathological conditions (Lamb et al., 1993; Pearson & Choi, 1993). However, the limited overexpression level achieved (two-fold) did not reproduce all the features of Alzheimer's disease (Murai et al., 1998) and triggered the generation of new YAC transgenic mice carrying mutant versions of the *APP* gene, and/or new candidate genes also associated with Alzheimer's disease (i.e. *apolipoprotein E4*, *presenilin-1* in Loring et al., 1996; Lamb et al., 1997, 1999).

YAC transgenic mice have also proven essential in the discovery of candidate genes responsible for the complex abnormal phenotype found in Down's syndrome patients. A panel of YAC transgenic mice was generated covering the human chromosome 21q22.2, a contiguous 2 Mb area known as the Down's syndrome region (Smith et al., 1995). A functional screening of

these transgenic mice led to the discovery of a gene (*minibrain*) implicated in learning defects associated with Down's syndrome (Smith et al., 1997a).

A number of informative and advantageous transgenic mice have been generated with YAC-based β -globin locus (i.e. Peterson et al., 1993; Gaensler et al., 1993; Peterson et al., 1996; Liu et al., 1997; Calzolari et al., 1999). Proper developmental expression patterns were demonstrated for the human β -globin transgenes, compared to the endogenous murine copies. In Table 1 these and other YAC transgenic animals are summarised in a comprehensive manner, emphasising the most relevant features of each set of experiments.

The generation of transgenic animals with YACs has been extended to other mammals with comparable success. To date, transgenic pigs (Yannoutsos et al., 1995), rabbits (Brem et al., 1996; Rouy et al., 1998), and rats (Fujiwara et al., 1997, 1999b) have been generated with YAC transgenes. In livestock, the benefits of YAC transgenesis are fundamentally focused in two fields: xenotransplantation and the efficient production of recombinant proteins of interest in the milk of transgenic animals.

Technical considerations for the generation of YAC transgenic animals

A number of different methods have been devised to produce transgenic animals with YACs both using pronuclear microinjection and transfection into ES cells (Schedl et al., 1993a; Jakobovits et al., 1993, 1999; Strauss et al., 1993; Choi et al., 1993; Gaensler et al., 1993; Huxley 1998; Peterson, 1999). A protocol suitable for the preparation of YAC DNA for pronuclear microinjection can be found at the following WEB page: <http://www.cnb.uam.es/~montoliu/prot.html>.

Most of the present experiments make use of standard pronuclear injection with the appropriate technical considerations for DNA molecules of this large size. Early reported methods were based on the modification of YAC vector arms to allow amplification of YACs inside the yeast cells in order to increase the recovery of YAC DNA molecules (Schedl et al., 1993a, 1996a). Soon it was obvious that the amplification step was not essential for adequate purification of YAC DNA and in subsequent updated methods this step was omitted (Peterson, 1997a; Hiemisch et al., 1998). Furthermore, the amplification step in yeast cells required the presence of *HSV thymidine kinase* gene in one of YAC-vector arms which has been reported to impair

Table 1. Transgenic animals generated with YACs

Transgene	Size (kb)	Animal	Aim	Human disease/condition	Reference
Mouse Tyrosinase	35, 100, 250	Mouse Rabbit	Rescue of albino phenotype Identification of regulatory sequences	Albinism	(Schedl et al., 1993b) (Montoliu et al., 1996) (Brem et al., 1996) (Jakobovits et al., 1993)
Human HPRT locus	670	Mouse	Molecular complementation of mutation		(Strauss et al., 1993)
Mouse $\alpha(1)$ collagen	150	Mouse	Molecular complementation of mutation		(Davies et al., 1993)
Human Ig light chain	300, 1300	Mouse	Production of human antibodies in mice		(Choi et al., 1993) (Zou et al., 1996)
Human Ig heavy chain	85	Mouse	Production of human antibodies in mice		(Green et al., 1994) (Fishwild et al., 1996) (Mendez et al., 1997)
Human Ig heavy and κ light chain	220, 170 800, 1020	Mouse	Production of human antibodies in mice		(Lamb et al., 1993, 1997) (Pearson and Choi, 1993) (Gaensler et al., 1993) (Peterson et al., 1993, 1995, 1996, 1998)
Human APP	400, 650	Mouse	Overexpression Model of human disease	Alzheimer	(Bungert et al., 1995, 1999) (Liu et al., 1997)
Human β -globin locus	248, 150	Mouse	Analysis of regulatory sequences	Down syndrome Thalassemias	(Navas et al., 1998) (Calzolari et al., 1999) (Tanimoto et al., 1999) (Frazer et al., 1995) (Rouy et al., 1998) (Acquati et al., 1999) (McCormick et al., 1995, 1997a)
Human Apolipoprotein (a)	270, 370	Mouse Rabbit	Pattern of expression and regulation	Atherosclerosis	(Smith et al., 1995, 1997)
Human Apolipoprotein B	108	Mouse Rabbit	Analysis of structure		(Huxley et al., 1996)
Human Chromosome 21 region 21q22.2	430-1100	Mouse	Mouse model of human disease	Down Syndrome	(Schedl et al., 1996b) (Yannoutsos et al., 1995, 1996) (Langford et al., 1996) (Manson et al., 1997) (Hiemisch et al., 1997) (Ainscough et al., 1997) (Chang et al., 1998)
Human PMP22	560	Mouse	Mouse model of human disease	Charcot-Marie-Tooth disease	
Human PAX6	420	Mouse	Mouse model for human disease	Aniridia	
Human MCP, CD59, CD46	420	Mouse	Xenotransplantation		
Human CFTR	320	Pig	Mouse model for human disease	Cystic fibrosis	
Human Hnf3 γ -lacZ	170	Mouse	Analysis of regulatory sequences		
Mouse H19, Igf2	130	Mouse	Genomic imprinting		
Human $\beta(S)$ -globin	240	Mouse	Mouse model for human disease	Sickle cell disease	

(Ainscough et al., 1991)
(Chang et al., 1998)

Sickle cell disease

Genomic imprinting
Mouse model for human disease

Mouse
Mouse

130
240

Mouse *H19*, *Igf2*
Human $\beta(S)$ -globin

Table 1. (continued)

Transgene	Size (kb)	Animal	Aim	Human disease/condition	Reference
Mouse <i>GATA-2</i>	120, 200 250	Mouse	Pattern of expression and regulation		(Zhou et al., 1998)
Mouse <i>GATA-3-lacZ</i>	120, 540 625	Mouse	Pattern of expression and regulation Identification of regulatory sequences		(Lakshmanan et al., 1998, 1999)
Human <i>WT1-lacZ</i>	280, 470	Mouse	Pattern of expression and regulation	Nephroblastomas	(Moore et al., 1998)
Human <i>SOX9-lacZ</i>	350	Mouse	Mouse model for human disease	Campomelic dysplasia	(Wunderle et al., 1998)
Human <i>Androgen Receptor-CAG</i> <i>Mouse Inversin</i>	450 450	Mouse	Mouse model for human disease Molecular complementation of mutation	X-linked spinal and bulbar muscular atrophy	(La Spada et al., 1998)
Mouse <i>downless</i>	200	Mouse	Mouse model for human disease		(Morgan et al., 1998)
Human Asthma QTL Human <i>IgH/c-myc</i> Human <i>presenilin-1</i> (PS-1) Human <i>DAZ</i>	400 240 1000 225	Mouse Mouse Mouse Mouse	Study of quantitative trait loci Mouse model for human disease Mouse model for human disease Molecular complementation of mutation	Autosomal hypohidrotic ectodermal dysplasia Asthma Burkitt's lymphoma Alzheimer Down Syndrome Spermatogenic defects	(Majumder et al., 1998) (Symula et al., 1999) (Butzler et al., 1997) (Palomo et al., 1999) (Lamb et al., 1999) (Slee et al., 1999)
Mouse <i>Xist/Xic</i>	430, 350 450	Mouse	X inactivation		(Heard et al., 1996) (Lee et al., 1999)
Human <i>XIST/XIC</i>	320, 460, 480	Mouse	X inactivation		(Matsuura et al., 1996) (Migeon et al., 1999) (Heard et al., 1999a,b)
Human α -lactalbumin Human <i>Huntingtin</i> Mouse <i>Myf-5</i> Human <i>Macrophage</i> <i>scavenger receptor</i> (MSR class A)	210 350, 600 680 180	Rat Mouse Mouse Mouse	Expression in mammary gland (hGH) Mouse model of human disease Analysis of regulatory sequences Analysis of gene function	Huntington disease Atherosclerosis	(Fujiwara et al., 1997, 1999a) (Hodgson et al., 1996, 1999) (Zweigerdt et al., 1997) (de Winther et al., 1999)
Mouse Olfactory receptors Human chromosome 5 5q31 cluster region Human <i>MJD1-CAG</i>	300 350-500 250	Mouse Mouse Mouse	Allelic inactivation Overexpression, discovery of new genes, genomic organisation Mouse model of human disease		(Ebrahimi et al., 2000) (Frazer et al., 1997) (Cemal et al., 1999)
				Spinocerebellar ataxia 3 (Machado-Joseph disease)	

germ-line transmission of YAC transgenes in males (Fujiwara et al., 1997).

Regarding the handling and microinjection of YAC DNA, the presence of ionic strength (100 mM sodium chloride) has been reported to be required to stabilise YAC DNA molecules in solution. Further, the addition of polyamines is recommended. These compacting agents promote the formation of YAC DNA-polyamine complexes and prevent shearing upon handling and microinjection of YACs (Montoliu et al., 1995; but see Bauchwitz & Constantini, 1998). Different strategies have been suggested to concentrate YAC DNA for microinjection including a second standard gel electrophoresis after preliminary isolation on PFGE (Schedl et al., 1993a); dialysis in sucrose (Gaensler et al., 1993) and the use of specific spin filtration units (Peterson et al., 1993). The use of two gels followed by an agarase treatment is preferred in some laboratories over the filtration alternative. The later, although easier and faster, requires special care to avoid potential breakage of YAC DNA molecules during the more extensive pipetting steps. Apart from the above mentioned protocols, suitable for the pronuclear microinjection of YACs, adapted versions have been developed for the efficient transfection of YACs into somatic (Compton et al., 1999) and mouse ES cells (Lee & Jaenisch, 1996; Bauchwitz & Constantini, 1998).

YACs are normally isolated from the rest of endogenous yeast chromosomes by preparative PFGE techniques (i.e. Schedl et al., 1993a). However, YACs are generally of the same size range as the endogenous yeast chromosomes. Thus, the isolation of YAC DNA by electrophoretic techniques can be impaired by the presence of comigrating or closely migrating endogenous yeast chromosomes. Some studies have shown that the cointegration of contaminant yeast endogenous chromosomes does not seem to have an overt effect in the expression of YAC transgenes (Jakobovits et al., 1993; Green et al., 1994; Mendez et al., 1997). However, it is always preferable to microinject YAC DNA samples free of contaminating yeast endogenous chromosomes. This can be easily achieved by mobilising the YAC to alternate yeast hosts with defined karyotypic alterations (Hamer et al., 1995). This new set of hosts, called yeast window strains, have been engineered using recombination-mediated chromosome fragmentation. Each strain has defined alterations in its karyotype, which provide an electrophoretic interval devoid of yeast endogenous chromosomes, thus allowing the isolation of relatively

pure YAC DNA regardless of YAC size (Hamer et al., 1995). All of the yeast window strains carry the *kar1-Δ15* mutation, thereby allowing the efficient transfer of a YAC from its original host into an appropriately selected yeast window strain using the *kar1*-transfer standard procedure (Spencer et al., 1994).

The overall efficiency of transgenesis with YACs (measured as the number of transgenic positive individuals found among newborns obtained) is comparable to that of standard DNA constructs although in some cases low efficiencies (<5%) are observed, most likely due to co-purified contaminants present in crude YAC DNA preparations. Although the usual number of YAC DNA molecules microinjected is much smaller than with standard plasmids (Brinster et al., 1985; Palmiter & Brinster, 1986), due to the bigger size of YAC transgenes, this does not seem to have an effect in transgenic efficiencies (Schedl et al., 1992, 1993b; Brem et al., 1996; Peterson, 1997a). The presence of vector sequences (10–15 kb long) found at either end of YACs does not appear to alter or prevent expression patterns of the borne transgenes (Schedl et al., 1992, 1993; Montoliu et al., 1996). Usually, 5–20% of newborn animals are found to be DNA-positive for the injected YAC transgene but only a variable proportion of them (20–70%) retains the entire YAC integrated in the host genome. Therefore, it is crucial to evaluate (i.e., by PCR) the integrity of YAC transgenes by analysing the presence of left and right YAC-vector arms, along with exhaustive Southern analysis with a set of internal probes, before subsequent experiments are actually carried out with selected founder animals (i.e. Smith et al., 1995; Montoliu et al., 1996; Brem et al., 1996; Peterson, 1997; Fujiwara et al., 1997). Additional methods that can be applied to assess YAC DNA integrity within the host genome include *recA*-assisted restriction endonuclease (RARE) analysis (Gnirke et al., 1993), restriction enzyme analysis with rare cutters (i.e. *SfiI*, *PpoI*; Peterson et al., 1998) and fiber fluorescence *in situ* hybridisation (FISH) (Rosenberg et al., 1995), a sophisticated method that uses stretched chromatin preparations to evaluate the integrity, organisation and copy number of integrated YAC transgenic sequences by FISH (Rosenberg et al., 1996).

Most transgenic animals generated with YACs carry single or few (<5) copies of transgenes integrated, in agreement with the limited number of DNA molecules that are microinjected. The presence of multiple copies (>5) is uncommon, but has been

repo
1995
A
germ
the s
et al
Gree
proa
allow
of Y
genic
ES c
both
tegra
selec
Hear
B
lems
cell
the
fecte
istan
or fo
bosy
hypo
(Jak
et al.
use
targe
gene
et al.
in a
Choi
YAC
miss
cases
woul
by p
gions
along
fere
borne
targe
S
ing
in th
able
geno
1993
some
ome

size (Hamer strains carrying the effinal host into strain using pincer et al.,

s with YACs positive indi- l) is compar- s although in served, most snt in crude usual number s much smal- r et al., 1985; e bigger size n to have an et al., 1992, /a). The pres- ng) found at ter or prevent enes (Schedl 96). Usually, to be DNA- e but only a etains the en- e. Therefore, the integrity sence of left th exhaustive l probes, be- y carried out r et al., 1995; 96; Peterson, methods that egrity within estriction en- t al., 1993), tters (i.e. *SfiI*, fluorescence et al., 1995), ed chromatin anisation and ic sequences

l with YACs ransgenes in- d number of The presence but has been

reported (i.e. Schedl et al., 1993b, 1996b; Smith et al., 1995; Ainscough et al., 1997; Moore et al., 1998).

Alternatively, the introduction of YACs into the germ-line of mice can be achieved via ES cells with the subsequent generation of chimaeric mice (Strauss et al., 1993; Choi et al., 1993; Jakobovits et al., 1993; Green et al., 1994; Mendez et al., 1997). This approach, although more difficult and time consuming allows the functional and structural characterisation of YAC-transgenes prior to the generation of transgenic mice (Green et al., 1994; Mendez et al., 1997). ES cell clones can be screened for the presence of both YAC-vector arms and different copy-number integration events, single and multicopy arrays, can be selected for further analysis in transgenic mice (i.e. Heard et al., 1999a,b).

Besides these advantages, some inherent problems should be taken into account before using ES-cell based approaches for the transfer of YACs into the germ-line of mice. First, YAC-positively transfected ES cell clones are selected for G418 resistance (Strauss et al., 1993; Choi et al., 1993) or for complementation of hypoxanthine phosphoribosyltransferase (HPRT)-deficient ES cell lines in hypoxanthine-aminopterin-thymidine (HAT) medium (Jakobovits et al. 1993; Green et al., 1994; Mendez et al., 1997). These selection procedures require the use of drug-selectable marker genes that are either targeted into a YAC vector arm (*neomycin resistance* gene in Strauss et al., 1993; HPRT gene in Green et al., 1994; Mendez et al., 1997) or co-transfected in a separate plasmid (*neomycin resistance* gene in Choi et al., 1993). The selection procedure biases YAC integration into 'open' or 'active' chromatin, permissive for marker gene expression. Thus, in these cases, position effects on YAC transgene expression would not be observed, or be effectively masked, by pre-selection for integration into euchromatic regions. Further, the co-integration of YAC transgenes along with drug-selectable marker genes might interfere with the normal expression pattern of the YAC-borne gene, as it has been reported in standard gene targeting approaches (Fiering et al., 1995).

Second, the fusion of yeast spheroblasts carrying YACs with HPRT-deficient ES cell lines results in the effective transfer and co-integration of variable and uncontrolled amounts of the remaining yeast genome into the ES cell genome (Jakobovits et al., 1993; Green et al., 1994; Mendez et al., 1997). In some ES cell clones, even the entire 12 Mb yeast genome was deduced to be present by fingerprint analysis

(Jakobovits et al., 1993). Surprisingly, adverse effects were not observed in transgenic mice derived from ES cell clones fused with yeast spheroblasts. However, it seems very unlikely to anticipate that none of the 6,000 genes known to be present in the yeast genome will not interfere, in some manner, with the complex gene expression programme of a mammalian cell. Therefore, the isolation of YAC DNA from yeast endogenous chromosomes prior to any gene transfer strategy should be, in our opinion, the recommended choice.

Transgenic animals generated with BACs/PACs

YACs are unique as vectors due to their huge cloning capacity and their unlimited potential for targeted modifications of incorporated genomic inserts (Schlessinger, 1990; Green et al., 1999). But, the routine work and handling of YACs requires specific skills and new expertise, which may not be present in all molecular biology laboratories. More importantly, several disadvantages are associated with YACs, including insert chimaerism (can be >50% of clones in a YAC library), insert instability, rearrangements and potential contamination with endogenous yeast chromosomes that can make difficult their efficient purification for microinjection or transfection into ES cells (Monaco & Larin, 1994; Green et al., 1999). To overcome these problems several other artificial chromosome-type vectors have been developed and have become popular. These include bacteriophage P1 clones (Sternberg et al., 1990, 1999; Pierce et al., 1992), bacterial artificial chromosomes (BACs) (Shizuya et al., 1992) and P1 bacteriophage-derived artificial chromosomes (PACs) (Ioannou et al., 1994). Figure 4 shows the basic modules from all artificial chromosomes-type vectors discussed in this review.

The bacteriophage P1 cloning system can efficiently accommodate 70–100 kb heterologous DNA inserts in *E. coli* (Sternberg 1999). P1 clones are usually derived from pAd10*sacBII*-type of vectors (Pierce et al., 1992). P1 clones are normally obtained by ligating genomic pieces of DNA with vector arms thereby generating a linear DNA molecule that is further processed and packaged in viral particles. Bacteriophage P1 particles are subsequently used to infect appropriate hosts where P1 clones are circularized by its *loxP*/Cre-recombinase system. Thereafter, P1 clones are subsequently maintained as single-copy circular plasmids. Copy-numbers, and hence DNA yield, can

be increased 10–30-fold before the DNA is isolated by regulating the activity of the bacteriophage P1 lytic replicon with IPTG. However, amplification of cloned DNA that contains repetitive sequences can lead to rearrangements (Birren et al., 1999). Upper limit size for inserts in P1 clones is fixed by the packaging capacity of bacteriophage particles (around 110 kb, including vector arms) (Sternberg 1999). PACs are very similar in structure to P1 clones (Figure 4, Ioannou et al., 1994; Birren et al., 1999). The main difference between PACs and P1 clones is that PACs lack the fixed upper size limit on insert length because PAC ligation mixes are transformed into their bacterial hosts by electroporation, whereas the generation of P1 clones involves the *in vitro* packaging step in bacteriophage particles (Sternberg, 1999; Birren et al., 1999). Thus, recombinant PACs achieve the same size range of inserts (100–300 kb) as do BACs (Ioannou et al., 1994; Monaco & Larin, 1994; Birren et al., 1999).

BACs, similar to PACs, are also circular plasmid DNA molecules that are hosted in *E. coli*. BACs can accommodate genomic inserts up to 300 kb and are derived from the F factor of *E. coli* (Shizuya et al., 1992). BAC vectors, such as pBeloBAC11, carry all sequences needed for autonomous replication, copy-number control and partitioning of the plasmid (Figure 4, Birren et al., 1999). In contrast to P1 clones and PACs, BACs are maintained as low-copy replicons and, correspondingly, yield lower quantities of DNA. For most applications, BACs and PACs are largely interchangeable and able to propagate large DNA inserts stably. Most protocols can be successfully applied to both types of clones, with the exception of antibiotic selection (kanamycin for PACs, chloramphenicol for BACs). Opposite to YACs, inserts cloned and maintained in BACs and PACs show low frequency (<5%) of chimaerism and much higher stability (Monaco & Larin, 1994; Birren et al., 1999). These new vectors have been used to generate genomic libraries that have been instrumental for most genome sequencing projects.

P1 bacteriophage clones were initially used to generate transgenic mice covering the human *apolipoprotein-B* gene (Linton et al., 1993), and the human chromosome 21q22.2, in combination with YACs, to isolate candidate genes associated with Down's syndrome (Smith et al., 1995). Furthermore, P1 clones and PACs have been successfully used to generate transgenic mice (McCormick et al., 1997b; Goodart et al., 1999; Chiu et al., 2000; Duff et al., 2000) and zebrafish (Jessen et al., 1999) in order to

study long-range genomic interactions with the help of reporter genes.

The use of BACs in transgenic experiments was first reported in 1997 (Yang et al., 1997). The authors describe a simple method to modify a BAC that was then transferred to the germ-line of mice. Since then, a number of reports using BACs for transgenesis have been published along with pioneer revisions in this subject (Dewar et al. 1997; Nielsen et al., 1999; Heintz, 2000; Camper & Saunders, 2000). Table 2 summarises the reported BAC and PAC transgenes to date.

Similar to the work previously undertaken with YACs, BACs have been applied in a wide variety of studies including: molecular complementation of mutations (Antoch et al., 1997; Probst et al., 1998), *in vivo* studies of gene function (Yu et al., 1999; Zuo et al., 1999), analysis of gene dosage (Antoch et al., 1997; Yang et al., 1999), and the identification and analysis of regulatory sequences found at long distances (Nielsen et al., 1997, 1998; Kaufman et al., 1999). Further, BACs have been evaluated for their potential to improve mammary gland transgenesis and for the production of recombinant proteins in the milk of transgenic animals (Stinnakre et al., 1999; Zuelke, 1998).

Technical considerations for the generation of BAC transgenic animals

Several methods have been devised to purify BAC DNA for mammalian transgenesis (Yang et al., 1997; Chrast et al., 1999). A protocol suitable for the preparation of BAC DNA for pronuclear microinjection can be found at the following WEB page: <http://www.med.umich.edu/tamc/BACDNA.html>. BACs have been microinjected in three different forms: circular supercoiled plasmid, linearised DNA and purified insert. It is possible to obtain transgenic animals with undigested BACs that carry essentially intact insert DNA (Antoch et al., 1997; Duff et al., 2000), but there is always a risk of obtaining undesirable DNA molecules generated by random linearisation within constructs prior to the integration. Therefore, the preferred methods have been to microinject linearised BAC clones (Antoch et al., 1997; Probst et al., 1998; Jessen et al., 1998) or, better, the isolation of genomic inserts by PFGE after suitable enzymatic release (normally *NotI*) from vector sequences (Yang et al., 1997; Kaufman et al., 1999; Stinnakre et al., 1999). In some

Table 3. YAC versus BAC/PAC transgenesis

	YACs	BACs/PACs
Host cell	<i>Saccharomyces cerevisiae</i>	<i>Escherichia coli</i>
Type of DNA molecule	Linear chromosome	Circular plasmid
Insert size	Up to 1–2 Mb	Up to 300 kb
Protocols for handling and isolation of DNA	Difficult	Easy
DNA yield	Low	Medium (BACs) High (PACs)
Resistance to shearing	Low (fragile)	High (in supercoiled form)
Direct sequencing possible	No	Yes
Selection markers in host cell	Complementation of auxotrophic mutants resistance to drugs	Resistance to antibiotics <i>kan^r</i> (PACs) <i>Cm^r</i> (BACs)
Insert chimaerism	High (>50% clones)	Very low (<5%)
Insert rearrangements	Yes	Very rare
Modification capabilities	Plenty	Few (increasing)
Protocols for Mutagenesis	Reproducible protocols Easy	Protocols in evolution Difficult

cases, such as generating transgenic mice with BACs carrying mouse DNA inserts, it is helpful to microinject linearised BAC molecules with vector sequences attached that can serve later as tags (polymorphisms) to identify the presence of transgenes. Alternately, the excision of genomic inserts from BACs based on pBe-loBAC11 vector derivatives by *NorI* digestion provides a few hundred vector base pairs at both ends (384 bp and 247 bp surrounding the *HindIII* cloning site) that can subsequently be used as tags or for PCR analysis. Similar to YACs, the presence of vector sequences in the microinjected BAC constructs does not seem to have an overt effect on the expression profiles of BAC transgenes, provided the size of the cloned genomic insert can accommodate most of the locus regulatory sequences (Kaufman et al., 1999).

When unique sites are not available the *recA*-assisted restriction endonuclease (RARE) technique may be employed to generate unique sites suitable for BAC insert excision (Boren et al., 1996; Nielsen et al., 1998; Nielsen et al., 1999). Alternatively, the presence of a unique *loxP* site in BAC/PAC-derived clones has been used for the effective Cre recombinase-mediated linearisation of transgenes before microinjection (Mullins et al., 1997).

BAC DNA can be efficiently stabilised in microinjection buffer by the addition of salt (usually sodium chloride) and polyamines, as reported for YACs

(Schedl et al., 1993a), although polyamines can be excluded without obvious effects, presumably due to the smaller size of BACs (Kaufman et al., 1999; Yang et al., 1997).

BAC transgenic animals have also been prepared via the ES cell route by co-transfection of BAC transgenes along with a selectable marker, followed by the production of chimaeric mice that are bred to create transgenic lines (Kaufman et al., 1999).

Transgenic animals carrying either BAC or PAC transgenes have been generated with comparable efficiencies to that of standard constructs (5–20% of newborn animals). As with YACs, most of BAC transgenic animals carry a limited number of integrated transgene copies (<5), but up to 13 copies of a BAC transgene have been reported (Nielsen et al., 1997). There are not many studies addressing copy number-dependent expression of BAC transgenes. Nevertheless, position-independent and copy-number-related expression has been shown in goat α -lactalbumin BAC transgenes in mice (Stinnakre et al., 1999).

Unfortunately, not all BAC/PAC transgenes integrate in the host genome as intact DNA molecules. Again, similar to YACs, rearrangements and insertion of fragmented transgenes can occur with BACs (Antoch et al., 1997; Kaufman et al., 1999) suggesting that rearrangement appears to be primarily related to trans-

gene
man
analy:
ded b
select
In
the op
YACs
Kaufn
ders,
prima
the ex
analys
genes
where
compa
is pres
problem

Modifi.

BACs a
purify t
method
commo
bacteria
In cont
ous met
with ye
their rap
laborato

In sj
have do
to their
which th
unlimite
duced in
the effici
ation sys
1994; Pei

In bac
been dev
BACs an
markably
(Yang et
1998; Jes
2000), op
of BAC/P
gree of co

gene size, irrespective of YAC or BAC origin (Kaufman et al., 1999). Therefore, detailed Southern/PCR analysis of integrated BAC transgenes is recommended before further experiments are undertaken with selected transgenic lines.

In conclusion, BAC/PAC constructs closely mimic the optimal performance in transgenesis achieved with YACs (McCormick & Nielsen, 1998; Huxley, 1998; Kaufman et al., 1999; Heintz, 2000; Camper & Saunders, 2000). The choice of vectors should be based primarily on the technical skills of the laboratory and the expected size of the expression domain to be analysed in transgenic experiments. Relatively small genes (<100 kb) can be analysed with BACs/PACs, whereas bigger loci require the use of YACs. A comparison of YAC versus BAC/PAC transgenesis is presented in Table 3, indicating the benefits and problems associated with both systems.

Modification of YAC/BAC/PAC transgenes

BACs and PACs are more convenient to propagate and purify than YACs because they do not require specific methods other than adaptations of existing protocols, commonly applied to routine work with plasmids in bacterial cells (Sternberg, 1999; Birren et al., 1999). In contrast, YACs require unique and more tedious methods along with the need to become familiar with yeast cells, features which might have prevented their rapid dissemination and implementation in some laboratories (Green et al., 1999).

In spite of such apparent disadvantages, YACs have documented advantages over BACs with regard to their modification capabilities and the ease with which these are achieved. The yeast system offers an unlimited variety of modifications that can be introduced in YACs using standard protocols that exploit the efficient yeast endogenous homologous recombination system (Schlessinger, 1990; Monaco & Larin, 1994; Peterson, 1997b; Green et al., 1999).

In bacteria, a number of innovative methods have been developed recently to retrofit specific markers in BACs and PACs (Mejia & Monaco, 1997) and, remarkably, for easier mutagenesis of BACs and PACs (Yang et al., 1997; Zhang et al., 1998; Nielsen et al., 1998; Jessen et al., 1998; Chiu et al., 2000; Yu et al., 2000), opening the possibility of extending the range of BAC/PAC transgenic approaches to a similar degree of complexity as that of YAC transgenics. In this

section we will discuss the modification potential of YACs compared to that of BACs and PACs.

Fragmentation vectors provide new telomeres along with selectable markers and have been used in YACs for a variety of purposes. These YAC vectors have been utilized to reduce the size of YAC constructs at precise locations, thereby generating a nested set of deletion derivatives (Montoliu et al., 1996; Wutz et al., 1997), to add selectable markers such as a neomycin-resistance gene (Lamb et al., 1993) or to combine size reduction with the addition of new features such as an amplification system (Schedl et al., 1993a; Fujiwara et al., 1997) or a reporter *lacZ* gene (Heard et al., 1996). Modified YACs are produced by targeted disruption of the original construct by homologous recombination driven in yeast cells. In some cases, homologous target sequences might not be known or available, but fragmentation can still be performed, at random locations, via repetitive sequence elements (i.e., B1 elements in the mouse genome; Zweigerdt et al., 1997; Lakshmanan et al., 1998). Following this approach, a YAC deletion series can be easily generated covering hundreds of kilobases in a single yeast transformation experiment, thus permitting the functional identification of distal regulatory elements (Zhou et al., 1998) or functional domains (Lee et al., 1999). With transgene DNA of human origin, an equivalent strategy can be devised using YAC fragmentation vectors that include *Alu* repeat sequences (Wunderle et al., 1998; Fujiwara et al., 1999b).

Modifications (i.e., deletions) at internal YAC sequences can also be performed by substituting the targeted sequence with a yeast selectable marker surrounded by neighbouring homologous sequences (Montoliu et al., 1996). Such YAC replacement vectors can also be used for the targeted insertion of heterologous sequences (Fujiwara et al., 1999a). However, these replacement type vectors leave behind the yeast selectable marker within the body of the YAC insert, which might interfere later with proper transgene expression. A cleaner alternative is to use the 'pop-in/pop-out' method in yeast cells, which uses an integrative type of plasmid vectors called YIP (yeast integrative plasmid). Two rounds of homologous recombination are required to substitute the original sequence of a YAC with the desired mutation, without retaining the selectable marker. This elegant and powerful approach has been used in a variety of cases: to introduce point mutations at precise locations within a YAC (Duff et al., 1994; McCormick et al., 1995, 1997a; Lamb et al., 1997), to reproduce

nines can be mably due to ., 1999; Yang

o been pre-nsfection of marker, fol-ic mice that ufman et al.,

BAC or PAC nparable effi-20% of new-AC transgenic ted transgene AC transgene 7). There are er-dependent less, position-xpression has transgenes in

isgenes integ-A molecules. and insertion 1 BACs (Ant-iggesting that lated to trans-

trinucleotide repeat characteristics of a set of human diseases (Hodgson et al., 1999; La Spada et al., 1998; Cemal et al., 1999), to generate minor deletions at regulatory sequences of the YAC-borne gene (Giraldo et al., 1999), and to introduce a reporter gene (i.e., *lacZ*) by transcriptional fusion with the targeted gene (Hiemisch et al., 1997; Ainscough et al., 1997) or via IRES-facilitated transcription (Vassaux & Huxley, 1997).

Studies of human β -globin locus gene regulation during development have benefitted largely from the introduction of precise mutations by homologous recombination techniques in yeast cells and the analysis of modified YAC transgenes in mice (Peterson et al., 1998). Some of the engineered modifications (i.e., amino-acid exchange, 5' breakpoints, internal deletions) correspond to equivalent defects found in patients affected by diverse forms of β -thalassemia syndromes and related human diseases (Peterson et al., 1995; Calzolari et al., 1999). Moreover, a large number of new mutations have been generated in human β -globin YAC transgenes to address the role of specific sequences (i.e. LCR) in the regulation of gene expression during development. These modifications include deletion of regulatory sequences (Peterson et al., 1996; Liu et al., 1997; Navas et al., 1998; Calzolari et al., 1999), inter-replacement of regulatory elements (Bungert et al., 1995) and alterations in the gene order or orientation of key regulatory sequences (Tanimoto et al., 1999).

The unlimited potential of YAC modifications is best illustrated by the generation of transgenic mice producing human antibodies (Mendez et al., 1997). In this report, the authors built megabase-sized YACs containing large contiguous genomic fragments corresponding to unarranged human heavy and light immunoglobulin genes using homologous recombination in yeast. Final YAC constructs were obtained by step-wise fusion, via recombination, of smaller and partially overlapping YACs (Mendez et al., 1997).

BACs have been more difficult to modify than YACs, although it is likely that the progressive development of new techniques in this rapidly evolving field will see greater ease of modification. The main reason for difficulties in modifying BACs and PACs is that bacterial host cells are *recA* deficient, to prevent undesired recombination and rearrangements and to favour insert stability. Thus, several recombination pathways in bacteria have been explored that are normally absent in BAC host cells. Their utilization requires either the transfer of the BAC to a suitable

bacterial strain or the exposure of the BAC-containing cell to the specific recombination machinery. Often these systems are not understood well and are prone to producing unexpected alterations that might give rise to the generation of erroneously modified BAC/PAC clones. Detailed structural analysis of resulting BAC clones is strongly recommended for the following methods.

The first describing a BAC modification system by homologous recombination in bacterial cells was that of Yang et al., (1997). Their approach is analogous to the yeast '*pop-in/pop-out*' strategy but is more complex, and has been used to target the incorporation of an IRES-*lacZ* reporter gene at precise internal locations within a BAC transgene. The expected homologous recombination event occurred at frequencies lower than those found in equivalent experiments with YAC constructs (Yang et al., 1997). This modification approach, based on the transient expression of *recA* protein, has been reproduced by several independent groups with BACs and P1 clones (Yu et al., 1999; Zuo et al., 1999; Payne et al., 1999). A simpler alternative was developed that uses the capacity of two properly oriented short DNA sequences (Chi-sites) to trigger the transfer of a DNA fragment located between them to homologous DNA by means of the *recBCD* pathway (Jessen et al., 1998). The authors showed that most BAC modified clones incorporated the desired homologous recombination event (a transcriptional fusion of a *lacZ* reporter gene) in the generation of transgenic zebrafish (Jessen et al., 1998). The same team reproduced chi-stimulated homologous recombination using a PAC clone with similar success (Jessen et al., 1999). Further experiments are necessary before the potential of this promising technique can be established.

A third modification system has been developed based on the *recE* and *recT* recombination pathway (Zhang et al., 1998). This approach, known as 'ET-cloning' uses homologous recombination driven by short sequences common to the BAC/PAC and the targeting vector. The authors showed the efficient modification of a P1 clone by targeting the insertion of an antibiotic resistance gene (Zhang et al., 1998). An updated version of the 'ET-cloning' method specially suited for the modification of BACs was recently reported, based on the functional counterparts of *recE* and *recT* proteins of bacteriophage λ (Muyrers et al., 1999). The combination of homologous recombination techniques coupled with the use of *FRT/FLP* and *loxP/CRE* site-specific recombination systems allows

the ex
detect
(Buchh
et al., 1
'ET-cl
in a nu
desired
that we
solution
protein
inducit
pressio
ments
proteco
could e
marker
an EGI
rying t
The ta
volved
(up to
with a
(WAP)
insertio
gene (
(Aguir
tentia
demon
lows th
ing bel
2000),
plied i
resista
ive an
of hon
one of
been i
in a B/
An
recentl
clones
provid
Other
recom
cludin
5' and
1998),
using
1999).
Fir
conve

BAC-containing machinery. Often and are prone to might give rise to modified BAC/PAC resulting BAC the following

modification system material cells was approach is ana-strategy but is to target the in-gene at precise isgene. The ex-ent occurred at an equivalent ex-g et al., 1997). on the transient reproduced by 's and P1 clones ne et al., 1999). that uses the ca-DNA sequences DNA fragment DNA by means al., 1998). The ed clones incor-mbination event orter gene) in the sen et al., 1998). ulated homolog-one with similar her experiments f this promising

been developed ination pathway known as 'ET-iation driven by AC/PAC and the ed the efficient g the insertion of et al., 1998). An ethod specially was recently re-terparts of *recE* (Muyrers et al., gous recombina-of *FRT/FLP* and n systems allows

the excision of the selectable marker employed to detect the very rare homologous recombination event (Buchholz et al., 1996; Zhang et al., 1998; Muyers et al., 1999). Despite the elegance and potential of the 'ET-cloning' method it has proven difficult to master in a number of laboratories that failed to obtain the desired BAC modification at the expected frequencies that were reported by the original authors. A possible solution was provided by placing all recombinogenic proteins under the control of a tightly regulated and inducible promoter, diminishing the risk of overexpression and the appearance of unwanted rearrangements (Narayanan et al., 1999). Using this modified protocol, called 'GET recombination', the authors could efficiently target the integration of a selectable marker (Narayanan et al., 1999), and the insertion of an EGFP reporter cassette within a 200 kb BAC carrying the human β -globin locus (Orford et al., 2000). The targeted insertion of the EGFP cassette also involved a series of deletions within the β -globin locus (up to 44 kb). Comparable results have been obtained with a BAC containing the mouse *whey-acidic protein* (*WAP*) gene that has been modified by the targeted insertion of a reporter secreted alkaline phosphatase gene (SEAP, Clontech) using 'GET recombination' (Aguirre & Montoliu, unpublished). The unlimited potential of the 'GET recombination' technique has been demonstrated by a recently developed method that allows the introduction of point mutations without leaving behind any operational sequences (Nefedov et al., 2000), analogous to the '*pop-in/pop-out*' strategies applied in YACs and BACs. In this case, the tetracycline resistance gene (*TetR*) has been used for both positive and negative selection in two consecutive rounds of homologous recombination. Using this technique, one of the most common β -thalassaemia mutations has been introduced into the intact β -globin locus present in a BAC (Nefedov et al., 2000).

Another modification system has been proposed recently for the targeted mutagenesis of BAC/PAC clones, based on the recombinogenic function provided by a defective λ prophage (Yu et al., 2000). Other mutagenesis methods, not using homologous recombination techniques, have been evaluated including the use of RARE cleavage in order to generate 5' and 3' BAC/PAC deletion derivatives (Nielsen et al., 1998), and the use of random-insertion mutagenesis using a transposon-mediated system (Brune et al., 1999).

Finally, YAC-shuttle vectors have been applied to convert P1 clones and PACs into YACs, to facilitate

further modification using the yeast homologous recombination system (Chiu et al., 2000; Poorkaj et al., 2000). Conversely, methods have been established to transform standard linear YACs (up to 250 kb) into circular YACs that can also be propagated in *E. coli* as BACs, thereby facilitating sequencing and functional analysis of genomic regions (Cocchia et al., 2000).

Perspectives

Despite the differences between YAC and BAC/PAC transgenesis approaches, both are associated with optimal performance in transgenic experiments. The size of their genomic inserts, ranging from less than 100 kb to more than 1 Mb, normally guarantees the inclusion of most regulatory sequences that are relevant for the faithful regulation of a gene. Therefore, artificial chromosome-type transgenes are usually expressed in appropriate spatial- and temporal-specific manners.

At present, YACs are much easier to modify than BACs/PACs, though more difficult to handle. The ability to easily retrofit BAC/PAC clones is a great challenge for the immediate future. Nonetheless, due to the relevance of BACs and PACs in genome research and functional genomics, it is likely that this situation will change, once established and reproducible protocols are disseminated within the scientific community. Artificial chromosome transgenesis has been fundamental for the isolation of candidate genes by complementation of mutations or alterations in the phenotype, and the generation of improved animal models of human genetic diseases. Large contiguous chromosomal fragments can be functionally scanned by transgenic approaches using a set of overlapping YAC/BAC/PAC clones spanning the region (i.e. Smith & Rubin, 1997; Frazer et al., 1997; Zhu et al., 2000). The identification of genes associated with known quantitative trait loci (QTL) has also been shown to benefit from artificial chromosome transgenesis (Symula et al., 1999). These techniques are expected to have a major impact on the analysis of gene expression and function in systems with a high degree of complexity, such as the mammalian central nervous system, in which standard loss-of-function approaches usually do not provide a clue to understand the role of a gene. In this respect, gene dosage experiments produced by increasing overexpression of transgenes within YAC/BAC/PAC clones will be instrumental for the correct understanding of gene function (Heintz, 2000).

Finally, the benefits of artificial chromosome transgenesis will be exported to biotechnological applications, such as the production of pharmaceutical or nutraceutical proteins in the mammary gland of transgenic animals (Zuelke, 1998; Fujiwara et al., 1999a).

In 1993, one of the first reviews discussing the future potential of artificial chromosome transgenesis concluded, with some degree of prudence, that 'bigger is probably better' (Forget, 1993). Indeed, seven years later, after having witnessed the enormous development of these techniques one can undoubtedly state: 'size matters' in animal transgenesis.

Note added in proof

Since submission and acceptance of this review two alternative methods have been described for engineering BACs by homologous recombination [Laloti and Heath (2001) *Nucleic Acids Res.* 29: e14; Swaminathan et al. (2001) *Genesis* 29: 14–21].

Acknowledgements

The authors are grateful to Alina Aguirre, Alfonso Lavado, Francina Langa and Glen Jeffery for critical reading of the manuscript and Thom Saunders for his BAC DNA purification protocol. This work has been supported by funds from CICYT (Bio97-0628), CAM (07B/0031/98), AEIC and Laboratorios Dr. Esteve, S.A.

References

- Acquati F, Hammer R, Ercoli B, Mooser V, Tao R, Ronicke V et al. (1999) Transgenic mice expressing a human apolipoprotein[a] allele. *J Lipid Res* 40: 994–1006.
- Ainscough JF, Koide T, Tada M, Barton S and Surani MA (1997) Imprinting of Igf2 and H19 from a 130 kb YAC transgene. *Development* 124: 3621–3632.
- Alami R, Greally JM, Tanimoto K, Hwang S, Feng YQ, Engel JD et al. (2000) Beta-globin YAC transgenes exhibit uniform expression levels but position effect variegation in mice. *Hum Mol Genet* 9: 631–636.
- Allan CM, Walker D and Taylor JM (1995) Evolutionary duplication of a hepatic control region in the human apolipoprotein E gene locus. Identification of a second region that confers high level and liver-specific expression of the human apolipoprotein E gene in transgenic mice. *J Biol Chem* 270: 26278–26281.
- Antoch MP, Song EJ, Chang AM, Vitaterna MH, Zhao Y, Wilsbacher LD et al. (1997) Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell* 89: 655–667.
- Asselbergs FA, Grossenbacher R, Ortmann R, Hengerer B, McMaster GK, Sutter E et al. (1998) Position-independent expression of a human nerve growth factor-luciferase reporter gene cloned on a yeast artificial chromosome vector. *Nucleic Acids Res* 26: 1826–1833.
- Bartolomei MS, Webber AL, Brunkow ME and Tilghman SM (1993) Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. *Genes Dev* 7: 1663–1673.
- Bauchwitz R and Costantini F (1998) YAC transgenesis: a study of conditions to protect YAC DNA from breakage and a protocol for transfection. *Biochim Biophys Acta* 1401: 21–37.
- Beermann F, Ruppert S, Hummler E, Bosc FX, Muller G, Ruther U et al. (1990) Rescue of the albino phenotype by introduction of a functional tyrosinase gene into mice. *EMBO J* 9: 2819–2826.
- Bender MA, Michael Bulger, Jennie Close, and Mark Groudine (2000) β -globin gene switching and DNase I sensitivity of the endogenous β -globin locus in mice do not require the locus control region. *Mol Cell* 5: 387–393.
- Birren B, Mancino V and Shizuya H (1999) Bacterial artificial chromosomes. In: Green ED, Birren B, Klapholz S, Myers RM and Hieter P (eds) *Genome Analysis. A Laboratory Manual* (vol III, Cloning Systems) (pp. 241–295) Cold Spring Harbor Laboratory Press, USA.
- Boren J, Lee I, Callow MJ, Rubin EM and Innerarity TL (1996) A simple and efficient method for making site-directed mutants, deletions, and fusions of large DNA such as P1 and BAC clones. *Genome Res* 6: 1123–1130.
- Brem G, Besenfelder U, Aigner B, Muller M, Liebl I, Schutz G et al. (1996) YAC transgenesis in farm animals: rescue of albinism in rabbits. *Mol Reprod Dev* 44: 56–62.
- Brinster RL, Chen HY, Trumbauer ME, Yagle MK and Palmiter RD (1985) Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc Natl Acad Sci USA* 82: 4438–4442.
- Brinster RL, Allen JM, Behringer RR, Gelinis RE and Palmiter RD (1988) Introns increase transcriptional efficiency in transgenic mice. *Proc Natl Acad Sci USA* 85: 836–840.
- Brune W, Menard C, Hobom U, Odenbreit S, Messerle M and Koszinowski UH (1999) Rapid identification of essential and nonessential herpesvirus genes by direct transposon mutagenesis. *Nat Biotechnol* 17: 360–364.
- Buchholz F, Angrand PO and Stewart AF (1996) A simple assay to determine the functionality of Cre or FLP recombination targets in genomic manipulation constructs. *Nucleic Acids Res* 24: 3118–3119.
- Bungert J, Dave U, Lim KC, Lieu KH, Shavit JA, Liu Q et al. (1995) Synergistic regulation of human beta-globin gene switching by locus control region elements HS3 and HS4. *Genes Dev* 9: 3083–3096.
- Bungert J, Tanimoto K, Patel S, Liu Q, Fear M and Engel JD (1999) Hypersensitive site 2 specifies a unique function within the human beta-globin locus control region to stimulate globin gene transcription. *Mol Cell Biol* 19: 3062–3072.
- Burke DT, Carle GF and Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 236: 806–812.
- Butzler C, Zou X, Popov AV and Bruggemann M (1997) Rapid induction of B-cell lymphomas in mice carrying a human IgH/c-mycYAC. *Oncogene* 14: 1383–1388.
- Calzolari R, McMorro T, Yannoutsos N, Langeveld A and Grosveld F (1999) Deletion of a region that is a candidate for the difference between the deletion forms of hereditary persistence of fetal hemoglobin and deltabeta-thalassemia affects beta- but not gamma-globin gene expression. *EMBO J* 18: 949–958.

- gene cloned
Nucleic Acids Res 26:
- Highman SM
Introducing of the
- s: a study of
d a protocol
- or G, Ruther
introduction
J 9: 2819-
- ork Groudine
ity of the en-
locus control
- rtificial chro-
mers RM and
nual (vol III,
or Laboratory
- y TL (1996)
cted mutants,
BAC clones.
- chutzh G et al.
of albinism in
- l Palmiter RD
ucing foreign
Acad Sci USA
- l Palmiter RD
in transgenic
- sserle M and
essential and
son mutagen-
- simple assay
mbination tar-
Acids Res 24:
- , Liu Q et al.
n gene switch-
34. *Genes Dev*
- ngel JD (1999)
within the hu-
te globin gene
- g of large seg-
s of artificial
- (1997) Rapid
human IgH/c-
- geveld A and
is a candidate
of hereditary
thalassemia af-
n. *EMBO J* 18:
- Camper SA and Saunders TL (2000) Transgenic rescue of mutant phenotypes using large DNA fragments. In: Accili D (ed). *Genetic Manipulation of Receptor Expression and Function*. (pp. 1-22) Wiley, New York.
- Cemal CK, Huxley C and Chamberlain S (1999) Insertion of expanded CAG trinucleotide repeat motifs into a yeast artificial chromosome containing the human Machado-Joseph disease gene. *Gene* 236: 53-61.
- Chang JC, Lu R, Lin C, Xu SM, Kan YW, Porcu S et al. (1998) Transgenic knockout mice exclusively expressing human hemoglobin S after transfer of a 240-kb beta-globin yeast artificial chromosome: A mouse model of sickle cell anemia. *Proc Natl Acad Sci USA* 95: 14886-14890.
- Chiu CH, Amemiya CT, Carr JL, Bhargava J, Hwang JK, Shashikant CS et al. (2000) A recombinogenic targeting method to modify large-inserts for cis-regulatory analysis in transgenic mice: construction and expression of a 100-kb, zebrafish *hoxa-11b-lacZ* reporter gene. *Dev Genes Evol* 210: 105-109.
- Choi T, Huang M, Gorman C and Jaenisch R (1991) A generic intron increases gene expression in transgenic mice. *Mol Cell Biol* 6: 3070-3074.
- Choi TK, Hollenbach PW, Pearson BE, Ueda RM, Weddell GN, Kurahara CG et al. (1993) Transgenic mice containing a human heavy chain immunoglobulin gene fragment cloned in a yeast artificial chromosome. *Nat Genet* 4: 117-123.
- Chrast R, Scott HS and Antonarakis SE (1999) Linearization and purification of BAC DNA for the development of transgenic mice. *Transgenic Res* 8: 147-150.
- Chung JH, Whiteley M and Felsenfeld G (1993) A 5' element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila*. *Cell* 74: 505-514.
- Clark AJ (1997) Transgene rescue. In: Houdebine LM (ed.) *Transgenic Animals, Generation and Use*. (pp. 267-272) Harwood Academic Publishers, The Netherlands.
- Cocchia M, Kouprina N, Kim SJ, Larionov V, Schlessinger D and Nagaraja R (2000) Recovery and potential utility of YACs as circular YACs/BACs. *Nucleic Acids Res* 28: E81.
- Compton ST, Henning KA, Chen M, Mansoura MK and Ashlock MA (1999) An improved method for routine preparation of intact artificial chromosome DNA (340-1000 kb) for transfection into human cells. *Nucleic Acids Res* 27: 1762-1765.
- Davies NP, Rosewell IR, Richardson JC, Cook GP, Neuberger MS, Brownstein BH et al. (1993) Creation of mice expressing human antibody light chains by introduction of a yeast artificial chromosome containing the core region of the human immunoglobulin kappa locus. *Biotechnology (NY)* 11: 911-914.
- Dewar K, Birren BW and Abderrahim H (1997) Bacterial artificial chromosomes and animal transgenesis. In: Houdebine LM (ed.) *Transgenic Animals, Generation and Use*. (pp. 283-287) Harwood Academic Publishers, The Netherlands.
- Dillon N and Grosveld F (1994) Chromatin domains as potential units of eukaryotic gene function. *Curr Opin Genet Dev* 4: 260-264.
- de Winther MP, van Dijk KW, van Vlijmen BJ, Gijbels MJ, Heus JJ, Wijers ER et al. (1999) Macrophage specific overexpression of the human macrophage scavenger receptor in transgenic mice, using a 180-kb yeast artificial chromosome, leads to enhanced foam cell formation of isolated peritoneal macrophages. *Atherosclerosis* 147: 339-347.
- Duff K, McGuigan A, Huxley C, Schulz F and Hardy J (1994) Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human amyloid precursor protein gene. *Gene Ther* 1: 70-75.
- Duff K, Knight H, Refolo LM, Sanders S, Yu X, Picciano M et al. (2000) Characterization of pathology in transgenic mice overexpressing human genomic and cDNA tau transgenes. *Neurobiol Dis* 7: 87-98.
- Ebrahimi FA, Edmondson J, Rothstein R and Chess A (2000) YAC transgene-mediated olfactory receptor gene choice. *Dev Dyn* 217: 225-231.
- Elgin SCR (1990) Chromatin structure and gene activity. *Curr Opin Cell Biol* 2: 437-445.
- Elson DA and Bartolomei MS (1997) A 5' differentially methylated sequence and the 3'-flanking region are necessary for H19 transgene imprinting. *Mol Cell Biol* 17: 309-317.
- Epner E, Reik A, Cimbora D, Telling A, Bender MA, Fiering S et al. (1998) The beta-globin LCR is not necessary for an open chromatin structure or developmentally regulated transcription of the native mouse beta-globin locus. *Mol Cell* 2: 447-455.
- Fan J, Wang J, Bensadoun A, Lauer SJ, Dang Q, Mahley RW et al. (1994) Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc Natl Acad Sci USA* 91: 8724-8728.
- Fiering S, Epner E, Robinson K, Zhuang Y, Telling A, Hu M et al. (1995) Targeted deletion of 5'HS2 of the murine beta-globin LCR reveals that it is not essential for proper regulation of the beta-globin locus. *Genes Dev* 9: 2203-2213.
- Fishwild DM, O'Donnell SL, Bengoechea T, Hudson DV, Harding F, Bernhard SL et al. (1996) High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice. *Nat Biotechnol* 14: 845-851.
- Forget BG (1993) YAC transgenes: bigger is probably better. *Proc Natl Acad Sci USA* 90: 7909-7911.
- Frazer KA, Narla G, Zhang JL and Rubin EM (1995) The apolipoprotein(a) gene is regulated by sex hormones and acute-phase inducers in YAC transgenic mice. *Nat Genet* 9: 424-431.
- Frazer KA, Ueda Y, Zhu Y, Gifford VR, Garofalo MR, Mohandas N et al. (1997) Computational and biological analysis of 680 kb of DNA sequence from the human 5q31 cytokine gene cluster region. *Genome Res* 7: 495-512.
- Fujiwara Y, Miwa M, Takahashi R, Hirabayashi M, Suzuki T and Ueda M (1997) Position-independent and high-level expression of human alpha-lactalbumin in the milk of transgenic rats carrying a 210-kb YAC DNA. *Mol Reprod Dev* 47: 157-163.
- Fujiwara Y, Miwa M, Takahashi R, Kodaira K, Hirabayashi M, Suzuki T et al. (1999a) High-level expressing YAC vector for transgenic animal bioreactors. *Mol Reprod Dev* 52: 414-420.
- Fujiwara Y, Takahashi RJ, Miwa M, Kameda M, Kodaira K, Hirabayashi M et al. (1999b) Analysis of control elements for position-independent expression of human alpha-lactalbumin YAC. *Mol Reprod Dev* 54: 17-23.
- Gaensler KM, Kitamura M and Kan YW (1993) Germ-line transmission and developmental regulation of a 150-kb yeast artificial chromosome containing the human beta-globin locus in transgenic mice. *Proc Natl Acad Sci USA* 90: 11381-11385.
- Ganss R, Montoliu L, Monaghan AP and Schutz G (1994) A cell-specific enhancer far upstream of the mouse tyrosinase gene confers high level and copy number-related expression in transgenic mice. *Embo J* 13: 3083-3093.
- Giraldo P, Gimenez E and Montoliu L (1999) The use of yeast artificial chromosomes in transgenic animals: expression studies of the tyrosinase gene in transgenic mice. *Genet Anal* 15: 175-178.
- Gnirke A, Huxley C, Peterson K and Olson MV (1993) Microinjection of intact 200- to 500-kb fragments of YAC DNA into mammalian cells. *Genomics* 15: 659-667.

- Goodart SA, Huynh C, Chen W, Cooper AD and Levy-Wilson B (1999) Expression of the human cholesterol 7 α -hydroxylase gene in transgenic mice. *Biochem Biophys Res Commun* 266: 454-459.
- Green ED, Hieter P and Spencer FA (1999) Yeast artificial chromosomes. In: Green ED, Birren B, Klapholz S, Myers RM and Hieter P (eds) *Genome Analysis. A Laboratory Manual* (vol III, Cloning Systems) (pp. 297-565) Cold Spring Harbor Laboratory Press, USA.
- Green LL, Hardy MC, Maynard-Currie CE, Tsuda H, Louie DM, Mendez MJ et al. (1994) Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat Genet* 7: 13-21.
- Grosveld F, van Assenfeldt GB, Greaves D and Kollias G (1987) Position-independent, high-level expression of the human beta-globin gene in transgenic mice. *Cell* 51: 975-985.
- Gutierrez-Adan A and Pintado B (2000) Effect of flanking matrix regions on the expression of microinjected transgenes during preimplantation development of mouse embryos. *Transgenic Res* 9: 81-89.
- Hamer L, Johnston M and Green ED (1995) Isolation of yeast artificial chromosomes free of endogenous yeast chromosomes: construction of alternate hosts with defined karyotypic alterations. *Proc Natl Acad Sci USA* 92: 11706-11710.
- Heard E, Kress C, Mongelard F, Courtier B, Rougeulle C, Ashworth A et al. (1996) Transgenic mice carrying an Xist-containing YAC. *Hum Mol Genet* 5: 441-450.
- Heard E, Mongelard F, Arnaud D, Chureau C, Vourc'h C and Avner P (1999a). Human XIST yeast artificial chromosome transgenes show partial X inactivation center function in mouse embryonic stem cells. *Proc Natl Acad Sci USA* 96: 6841-6846.
- Heard E, Mongelard F, Arnaud D and Avner P (1999b) Xist yeast artificial chromosome transgenes function as X-inactivation centers only in multicopy arrays and not as single copies. *Mol Cell Biol* 19: 3156-3166.
- Heintz N (2000) Analysis of mammalian central nervous system gene expression and function using bacterial artificial chromosome-mediated transgenesis. *Hum Mol Genet* 9: 937-943.
- Hiemisch H, Schutz G and Kaestner KH (1997) Transcriptional regulation in endoderm development: characterization of an enhancer controlling Hnf3g expression by transgenesis and targeted mutagenesis. *EMBO J* 16: 3995-4006.
- Hiemisch H, Umland T, Montoliu L and Schutz G (1998) The generation of transgenic mice with yeast artificial chromosomes. In: Cid-Arregui A and García-Carrancá A (eds) *Microinjection and Transgenesis, Strategies and Protocols* (pp. 297-308) Springer-Verlag, Berlin Heidelberg.
- Hodgson JG, Smith DJ, McCutcheon K, Koide HB, Nishiyama K, Dinulos MB et al. (1996) Human huntingtin derived from YAC transgenes compensates for loss of murine huntingtin by rescue of the embryonic lethal phenotype. *Hum Mol Genet* 5: 1875-1885.
- Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R et al. (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* 23: 181-192.
- Huxley C, Passage E, Manson A, Putzu G, Figarella-Branger D, Pellissier JF et al. (1996) Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum Mol Genet* 5: 563-569.
- Huxley C (1998) Exploring gene function: use of yeast artificial chromosome transgenesis. *Methods* 2: 199-210.
- Iannou PA, Amemiya CT, Ganes J, Kroisel PM, Shizuya H, Chen C et al. (1994) A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. *Nat Genet* 6: 84-89.
- Jakobovits A, Moore AL, Green LL, Vergara GJ, Maynard-Currie CE, Austin HA et al. (1993) Germ-line transmission and expression of a human-derived yeast artificial chromosome. *Nature* 362: 255-258.
- Jakobovits A (1994) YAC vectors. Humanizing the mouse genome. *Curr Biol* 4: 761-763.
- Jakobovits A, Lamb BT and Peterson KR (1999) Technical considerations for the generation of YAC transgenic animals. In: Tuan RS and Lo CW (eds), *Methods in Molecular Biology*, vol. 136: Developmental Biology Protocols, Vol. II, (pp. 435-453) Humana Press, Totowa, N.J.
- Jessen JR, Meng A, McFarlane RJ, Paw BH, Zon LI, Smith GR et al. (1998) Modification of bacterial artificial chromosomes through chi-stimulated homologous recombination and its application in zebrafish transgenesis. *Proc Natl Acad Sci USA* 95: 5121-5126.
- Jessen JR, Willett CE and Lin S (1999) Artificial chromosome transgenesis reveals long-distance negative regulation of rag1 in zebrafish. *Nat Genet* 23: 15-16.
- Kaufman RM, Pham CT and Ley TJ (1999) Transgenic analysis of a 100-kb human beta-globin cluster-containing DNA fragment propagated as a bacterial artificial chromosome. *Blood* 94: 3178-3184.
- Kluppel M, Beermann F, Ruppert S, Schmid E, Hummler E and Schutz G (1991) The mouse tyrosinase promoter is sufficient for expression in melanocytes and in the pigmented epithelium of the retina. *Proc Natl Acad Sci USA* 88: 3777-3781.
- Kuhn RM and Ludwig RA (1994) Complete sequence of the yeast artificial chromosome cloning vector pYAC4. *Gene* 141: 125-127.
- La Spada AR, Peterson KR, Meadows SA, McClain ME, Jeng G, Chmela RS et al. (1998) Androgen receptor YAC transgenic mice carrying CAG 45 alleles show trinucleotide repeat instability. *Hum Mol Genet* 7: 959-967.
- Laemmli UK, Käs E, Poljak L and Adachi Y (1992) Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. *Curr Opin Genet Dev* 2: 275-285.
- Lakshmanan G, Lieu KH, Grosveld F and Engel JD (1998) Partial rescue of GATA-3 by yeast artificial chromosome transgenes. *Dev Biol* 204: 451-463.
- Lakshmanan G, Lieu KH, Lim KC, Gu Y, Grosveld F, Engel JD et al. (1999) Localization of distant urogenital system-, central nervous system-, and endocardium-specific transcriptional regulatory elements in the GATA-3 locus. *Mol Cell Biol* 19: 1558-1568.
- Lamb BT, Sisodia SS, Lawler AM, Slunt HH, Kitt CA, Kearns WG et al. (1993) Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice. *Nat Genet* 5: 22-30.
- Lamb BT and Gearhart JD (1995) YAC transgenics and the study of genetics and human disease. *Curr Opin Genet Dev* 5: 342-348.
- Lamb BT, Call LM, Slunt HH, Bardel KA, Lawler AM, Eckman CB et al. (1997) Altered metabolism of familial Alzheimer's disease-linked amyloid precursor protein variants in yeast artificial chromosome transgenic mice. *Hum Mol Genet* 6: 1535-1541.
- Lamb BA, Bardel KA, Kulnane LS, Anderson JJ, Holtz G, Wagner SL et al. (1999) Amyloid production and deposition in mutant amyloid precursor protein and presenilin-1 yeast artificial chromosome transgenic mice. *Nat Neurosci* 2: 695-697.

Lan
I
c
I
Lee
I
2
Lee
i
A
Li C
c
Li S
(
fi
d
Linte
et
ti-
h
Liu C
re
ba
m
Lori
et
di
re
18
Maju
O
m
Mans
Ej
wi
Matsu
ex
ch
McCo
Ye
ge-
tac
10
McCo
Se
Aq
ca
the
McCo
ust
als.
an
Ne
McCo
om
teir
10
McCr
and
tra
McKn
nig
pos
gen

- Shizuya H, Chen J (1997) A vector for the generation of transgenic animals. *Genet* 6: 84–91.
- Maynard-Currie CE (1997) Transgenic mice: a model for studying gene function and disease. *Nature* 387: 15–21.
- Technical review of transgenic animals. In: *Transgenic Animals*, vol. 1 (pp. 435–453).
- LI, Smith GR (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- al chromosome inactivation of ragl in transgenic mice. *Proc Natl Acad Sci USA* 95: 113–121.
- enic analysis of DNA fragment in transgenic mice. *Blood* 94: 3178–3184.
- Hummler E and Smith GR (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- is sufficient for the development of epithelium of the stomach. *Gene* 141: 125–131.
- lain ME, Jeng M (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- tor YAC transgenic mice. *Nucleic Acids Res* 25: 2539–2540.
- ucleotide repeat expansion in transgenic mice. *Hum Mol Genet* 5: 451–459.
- 1992) Scaffold-assembly of transgenic mice. *Genet Dev* 2: 1–10.
- D (1998) Partial inactivation of transgenes in transgenic mice. *Proc Natl Acad Sci USA* 95: 113–121.
- eld F, Engel JD (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- system-, centromeric and transcriptional control of gene expression in transgenic mice. *Cell Biol* 19: 1–10.
- CA, Kearns WG (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- re 400 kilobase transgenic mice. *Nat Genet* 15: 146–156.
- and the study of gene function. *Dev* 5: 342–348.
- LM, Eckman CB (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- heimer's disease-yeast artificial chromosome (YAC) transgenic mice. *Nat Genet* 15: 146–156.
- Holtz G, Waggoner D (1997) Transgenic mice: a model for studying gene function and disease. *Genet* 6: 84–91.
- deposition in transgenic mice. *Nat Genet* 15: 146–156.
- Langford GA, Cozzi E, Yannoutsos N, Lancaster R, Elsom K, Chen P et al. (1996) Production of pigs transgenic for human regulators of complement activation using YAC technology. *Transplant Proc* 28: 862–863.
- Lee JT and Jaenisch R (1996) A method for high efficiency YAC lipofection into murine embryonic stem cells. *Nucleic Acids Res* 24: 5054–5055.
- Lee JT, Lu N and Han Y (1999) Genetic analysis of the mouse X inactivation center defines an 80-kb multifunction domain. *Proc Natl Acad Sci USA* 96: 3836–3841.
- Li Q, Harju S and Peterson KR (1999) Locus control regions: coming of age at a decade plus. *Trends Genet* 15: 403–408.
- Li S, Hammer RE, George-Raizen JB, Meyers KC, Garrard WT (2000) High-level rearrangement and transcription of yeast artificial chromosome-based mouse Ig kappa transgenes containing distal regions of the contig. *J Immunol* 164: 812–824.
- Linton MF, Farese RV Jr, Chiesa G, Grass DS, Chin P, Hammer RE et al. (1993) Transgenic mice expressing high plasma concentrations of human apolipoprotein B100 and lipoprotein(a). *J Clin Invest* 92: 3029–3037.
- Liu Q, Bungert J and Engel JD (1997) Mutation of gene-proximal regulatory elements disrupts human epsilon-, gamma-, and beta-globin expression in yeast artificial chromosome transgenic mice. *Proc Natl Acad Sci USA* 94: 169–174.
- Loring JF, Paszty C, Rose A, McIntosh TK, Murai H, Pierce JE et al. (1996) Rational design of an animal model for Alzheimer's disease: introduction of multiple human genomic transgenes to reproduce AD pathology in a rodent. *Neurobiol Aging* 17: 173–182.
- Majumder K, Shawlot W, Schuster G, Harrison W, Elder FF and Overbeek PA (1998) YAC rescue of downless locus mutations in mice. *Mamm Genome* 9: 863–868.
- Manson AL, Trezise AE, MacVinish LJ, Kasschau KD, Birchall N, Episkopou V et al. (1997) Complementation of null CF mice with a human CFTR YAC transgene. *EMBO J* 16: 4238–4249.
- Matsuura S, Episkopou V, Hamvas R and Brown SD (1996) Xist expression from an Xist YAC transgene carried on the mouse Y chromosome. *Hum Mol Genet* 5: 451–459.
- McCormick SP, Ng JK, Taylor S, Flynn LM, Hammer RE and Young SG (1995) Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein(a). *Proc Natl Acad Sci USA* 92: 10147–10151.
- McCormick SP, Ng JK, Cham CM, Taylor S, Marcovina SM, Segrest JP et al. (1997a) Transgenic mice expressing human ApoB95 and ApoB97. Evidence that sequences within the carboxyl-terminal portion of human apoB100 are important for the assembly of lipoprotein. *J Biol Chem* 272: 23616–23622.
- McCormick SP, Allan CM, Taylor JM and Young SG (1997b) The use of P1 bacteriophage clones to generate transgenic animals. In: Houdebine LM (ed.) *Transgenic Animals, Generation and Use*. (pp. 273–281) Harwood Academic Publishers, The Netherlands.
- McCormick SP and Nielsen LB (1998) Expression of large genomic clones in transgenic mice: new insights into apolipoprotein B structure, function and regulation. *Curr Opin Lipidol* 9: 103–111.
- McCreath KJ, Howcroft J, Campbell KHS, Colman A, Schnieke AE and Kind AJ (2000) Production of gene-altered sheep by nuclear transfer from cultured somatic cells. *Nature* 405: 1066–1069.
- McKnight RA, Shamay A, Sankaran L, Wall RJ and Hennighausen L (1992) Matrix-attachment regions can impart position-independent regulation of a tissue-specific gene in transgenic mice. *Proc Natl Acad Sci USA* 89: 6943–6947.
- Mejia JE and Monaco AP (1997) Retrofitting vectors for *Escherichia coli*-based artificial chromosomes (PACs and BACs) with markers for transfection studies. *Genome Res* 7: 179–186.
- Mendez MJ, Green LL, Corvalan JR, Jia XC, Maynard-Currie CE, Yang XD et al. (1997) Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. *Nat Genet* 15: 146–156.
- Migeon BR, Kazi E, Haisley-Royster C, Hu J, Reeves R, Call L et al. (1999) Human X inactivation center induces random X chromosome inactivation in male transgenic mice. *Genomics* 59: 113–121.
- Monaco AP and Larin Z (1994) YACs, BACs, PACs and MACs: artificial chromosomes as research tools. *Trends Biotechnol* 12: 280–286.
- Montoliu L, Schedl A, Kelsey G, Lichter P, Larin Z, Lehrach H et al. (1993) Generation of transgenic mice with yeast artificial chromosomes. *Cold Spring Harb Symp Quant Biol* 58: 55–62.
- Montoliu L, Schedl A, Kelsey G, Zentgraf H, Lichter P and Schutz G (1994) Germ line transmission of yeast artificial chromosomes in transgenic mice. *Reprod Fertil Dev* 6: 577–584.
- Montoliu L, Bock CT, Schutz G and Zentgraf H (1995) Visualization of large DNA molecules by electron microscopy with polyamines: application to the analysis of yeast endogenous and artificial chromosomes. *J Mol Biol* 246: 486–492.
- Montoliu L, Umland T and Schutz G (1996) A locus control region at -12 kb of the tyrosinase gene. *EMBO J* 15: 6026–6034.
- Moore AW, Schedl A, McInnes L, Doyle M, Hecksher-Sorensen J and Hastie ND (1998) YAC transgenic analysis reveals Wilms' tumour 1 gene activity in the proliferating coelomic epithelium, developing diaphragm and limb. *Mech Dev* 79: 169–184.
- Morgan D, Tumpenny L, Goodship J, Dai W, Majumder K, Matthews L, Gardner A et al. (1998) Inversin, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the inv mouse. *Nat Genet* 20: 149–156.
- Mullins LJ, Kotelevtseva N, Boyd AC and Mullins JJ (1997) Efficient Cre-lox linearisation of BACs: applications to physical mapping and generation of transgenic animals. *Nucleic Acids Res* 25: 2539–2540.
- Murai H, Pierce JE, Raghupathi R, Smith DH, Saatman KE, Trojanowski JQ et al. (1998) Two-fold overexpression of human beta-amyloid precursor proteins in transgenic mice does not affect the neuromotor, cognitive, or neurodegenerative sequelae following experimental brain injury. *J Comp Neurol* 392: 428–438.
- Muyrers JP, Zhang Y, Testa G and Stewart AF (1999) Rapid modification of bacterial artificial chromosomes by ET-recombination. *Nucleic Acids Res* 27: 1555–1557.
- Narayanan K, Williamson R, Zhang Y, Stewart AF and Ioannou PA (1999) Efficient and precise engineering of a 200 kb beta-globin human/bacterial artificial chromosome in *E. coli* DH10B using an inducible homologous recombination system. *Gene Ther* 6: 442–447.
- Navas PA, Peterson KR, Li Q, Skarpidi E, Rohde A, Shaw SE et al. (1998) Developmental specificity of the interaction between the locus control region and embryonic or fetal globin genes in transgenic mice with an HS3 core deletion. *Mol Cell Biol* 18: 4188–4196.
- Nefedov M, Williamson R and Ioannou PA (2000) Insertion of disease-causing mutations in BACs by homologous recombination in *Escherichia coli*. *Nucleic Acids Res* 28: E79.
- Nielsen LB, McCormick SP, Pierotti V, Tam C, Gunn MD, Shizuya H et al. (1997) Human apolipoprotein B transgenic mice gen-

- erated with 207- and 145-kilobase pair bacterial artificial chromosomes. Evidence that a distant 5'-element confers appropriate transgene expression in the intestine. *J Biol Chem* 272: 29752-29758.
- Nielsen LB, Kahn D, Duell T, Weier HU, Taylor S and Young SG (1998) Apolipoprotein B gene expression in a series of human apolipoprotein B transgenic mice generated with recA-assisted endonuclease cleavage-modified bacterial artificial chromosomes. An intestine-specific enhancer element is located between 54 and 62 kilobases 5' to the structural gene. *J Biol Chem* 273: 21800-21807.
- Nielsen LB, McCormick SP and Young SG (1999) A new approach for studying gene regulation by distant DNA elements in transgenic mice. *Scand J Clin Lab Invest Suppl* 229: 33-39.
- Orford M, Nefedov M, Vadolas J, Zaibak F, Williamson R and Ioannou PA (2000) Engineering EGFP reporter constructs into a 200 kb human beta-globin BAC clone using GET recombination. *Nucleic Acids Res* 28: E84.
- Palmiter RD and Brinster RL (1986). Germ-line transformation of mice. *Annu Rev Genet* 20: 465-499.
- Palmiter RD, Sandgren EP, Avarbock MR, Allen DD and Brinster RL (1991) Heterologous introns can enhance expression of transgenes in mice. *Proc Natl Acad Sci USA* 88: 478-482.
- Palomo C, Zou X, Nicholson IC, Butzler C and Bruggemann M (1999) B-cell tumorigenesis in mice carrying a yeast artificial chromosome-based immunoglobulin heavy/c-myc translocus is independent of the heavy chain intron enhancer (Emu). *Cancer Res* 59: 5625-5628.
- Patapoutian A, Miner JH, Lyons GE and Wold B (1993) Isolated sequences from the linked Myf-5 and MRF4 genes drive distinct patterns of muscle-specific expression in transgenic mice. *Development* 118: 61-69.
- Payne CM, Mullins LJ and Mullins JJ (1999) Manipulating large genomic clones via *in vivo* recombination in bacteria. *J Hum Hypertens* 13: 845-848.
- Pearson BE and Choi TK (1993) Expression of the human beta-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. *Proc Natl Acad Sci USA* 90: 10578-10582.
- Peterson KR, Clegg CH, Huxley C, Josephson BM, Haugen HS, Furukawa T et al. (1993) Transgenic mice containing a 248-kb yeast artificial chromosome carrying the human beta-globin locus display proper developmental control of human globin genes. *Proc Natl Acad Sci USA* 90: 7593-7597.
- Peterson KR, Li QL, Clegg CH, Furukawa T, Navas PA, Norton EJ et al. (1995) Use of yeast artificial chromosomes (YACs) in studies of mammalian development: production of beta-globin locus YAC mice carrying human globin developmental mutants. *Proc Natl Acad Sci USA* 92: 5655-5659.
- Peterson KR, Clegg CH, Navas PA, Norton EJ, Kimbrough TG and Stamatoyannopoulos G (1996) Effect of deletion of 5'HS3 or 5'HS2 of the human beta-globin locus control region on the developmental regulation of globin gene expression in beta-globin locus yeast artificial chromosome transgenic mice. *Proc Natl Acad Sci USA* 93: 6605-6609.
- Peterson KR (1997a) Production and analysis of transgenic mice containing yeast artificial chromosomes. *Genet Eng* 19: 235-255.
- Peterson KR, Clegg CH, Li Q and Stamatoyannopoulos G (1997b) Production of transgenic mice with yeast artificial chromosomes. *Trends Genet* 13: 61-66.
- Peterson KR, Navas PA, Li Q and Stamatoyannopoulos G (1998) LCR-dependent gene expression in beta-globin YAC transgenics: detailed structural studies validate functional analysis even in the presence of fragmented YACs. *Hum Mol Genet* 7: 2079-2088.
- Peterson KR (1999) Use of yeast artificial chromosomes to express genes in transgenic mice. *Methods Enzymol* 306: 186-203.
- Pfeifer K, Leighton PA and Tilghman SM (1996) The structural H19 gene is required for transgene imprinting. *Proc Natl Acad Sci USA* 93: 13876-13883.
- Pierce JC, Sauer B and Sternberg N (1992) A positive selection vector for cloning high molecular weight DNA by the bacteriophage P1 system: Improved cloning efficiency. *Proc Natl Acad Sci USA* 89: 2056-2060.
- Poorkaj P, Peterson KR and Schellenberg GD (2000) Single-step conversion of P1 and P1 artificial chromosome clones into yeast artificial chromosomes. *Genomics* 68: 106-110.
- Porcu S, Kitamura M, Witkowska E, Zhang Z, Mutero A, Lin C et al. (1997) The human beta globin locus introduced by YAC transfer exhibits a specific and reproducible pattern of developmental regulation in transgenic mice. *Blood* 90: 4602-4609.
- Probst FJ, Fridell RA, Raphael Y, Saunders TL, Wang A, Liang Y et al. (1998) Correction of deafness in shaker-2 mice by an unconventional myosin in a BAC transgene. *Science* 280: 1444-1447.
- Rosenberg C, Florijn RJ, Van de Rijke FM, Blonden LA, Raap TK, Van Ommen GJ et al. (1995) High resolution DNA fiberfish on yeast artificial chromosomes: direct visualization of DNA replication. *Nat Genet* 10: 477-479.
- Rosenberg C, Voltz AK, Lawler AM, Lamb BT, Stetten G and Gearhart JD (1996) Alterations of yeast artificial chromosome transgenic sequences in stretched embryonic stem-cell chromatin visualized by fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 75: 67-70.
- Rouy D, Duverger N, Lin SD, Emmanuel F, Houdebine LM, Deneffe P et al. (1998) Apolipoprotein(a) yeast artificial chromosome transgenic rabbits. Lipoprotein(a) assembly with human and rabbit apolipoprotein B. *J Biol Chem* 273: 1247-1251.
- Schedl A, Beermann F, Thies E, Montoliu L, Kelsey G and Schutz G (1992) Transgenic mice generated by pronuclear injection of a yeast artificial chromosome. *Nucleic Acids Res* 20: 3073-3077.
- Schedl A, Larin Z, Montoliu L, Thies E, Kelsey G, Lehrach H et al. (1993a) A method for the generation of YAC transgenic mice by pronuclear microinjection. *Nucleic Acids Res* 21: 4783-4787.
- Schedl A, Montoliu L, Kelsey G and Schutz G (1993b) A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice. *Nature* 362: 258-261.
- Schedl A, Grimes B and Montoliu L (1996a) YAC transfer by microinjection. *Methods Mol Biol* 54: 293-306.
- Schedl A, Ross A, Lee M, Engelkamp DPR, van Heyninge V and Hastie ND (1996b) Influence of PAX6 gene dosage on development: overexpression causes severe eye abnormalities. *Cell* 86: 71-82.
- Schlessinger D (1990) Yeast artificial chromosomes: tools for mapping and analysis of complex genomes. *Trends Genet* 6: 248-258.
- Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y et al. (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proc Natl Acad Sci USA* 89: 8794-8797.
- Sippel AE, Saueressig H, Hubler MC, Faust N and Bonifer C (1997) Insulation of transgenes from chromosomal position effects. In: Houdebine LM (ed.) *Transgenic Animals, Generation and Use*. (pp. 267-272) Harwood Academic Publishers, The Netherlands.

- tional analysis
Mol Genet 7:
- mes to express
186–203.
- structural H19
Natl Acad Sci
- e selection vec-
bacteriophage
l Acad Sci USA
- 00) Single-step
clones into yeast
- o A, Lin C et al.
by YAC trans-
developmental
09.
- Wang A, Liang
ker-2 mice by
Science 280:
- iden LA, Raap
on DNA fiber-
ization of DNA
- Stetten G and
d chromosome
-cell chromatin
Cytogenet Cell
- ine LM, Dene-
al chromosome
th human and
1251.
- y G and Schutz
clear injection
Res 20: 3073–
- ehrach H et al.
sgenic mice by
4783–4787.
- 3b) A yeast ar-
e confers copy
e. *Nature* 362:
- AC transfer by
- eyninge V and
ge on develop-
ilities. *Cell* 86:
- nes: tools for
tends Genet 6:
- : T, Tachiiri Y
300-kilobase-
coli using an
8794–8797.
- onifer C (1997)
tion effects. In:
ation and Use.
ie Netherlands.
- Slee R, Grimes B, Speed RM, Taggart M, Maguire SM, Ross A
et al. (1999) A human DAZ transgene confers partial rescue of
the mouse Dazl null phenotype. *Proc Natl Acad Sci USA* 96:
8040–8045.
- Smith DJ, Zhu Y, Zhang J, Cheng JF and Rubin EM (1995) Con-
struction of a panel of transgenic mice containing a contiguous
2-Mb set of YAC/P1 clones from human chromosome 21q22.2.
Genomics 27: 425–434.
- Smith DJ, Stevens ME, Sudanagunta SP, Bronson RT, Makhinson
M, Watabe AM et al. (1997) Functional screening of 2 Mb of
human chromosome 21q22.2 in transgenic mice implicates min-
ibrain in learning defects associated with Down syndrome. *Nat
Genet* 16: 28–36.
- Smith DJ and Rubin EM (1997) Functional screening and complex
traits: human 21q22.2 sequences affecting learning in mice. *Hum
Mol Genet* 6: 1729–1733.
- Spencer F, Hugerat Y, Simchen G, Hurko O, Connelly C and Hieter
P (1994) Yeast *karl* mutants provide an effective method for
YAC transfer to new hosts. *Genomics* 22: 118–126.
- Sternberg N, Ruether J and deRiel K (1990) Generation of a 50,000-
member human DNA library with an average DNA insert size
of 75–100 kb in a bacteriophage P1 cloning vector. *New Biol* 2:
151–162.
- Sternberg N (1999) Cloning into bacteriophage P1 vectors. In:
Green ED, Birren B, Klapholz S, Myers RM and Hieter P
(eds) *Genome Analysis. A Laboratory Manual* (vol III, Cloning
Systems) (pp. 203–239) Cold Spring Harbor Laboratory Press,
USA.
- Stinnakre MG, Soulier S, Schibler L, Lepourry L, Mercier JC
and Vilotte JL (1999) Position-independent and copy-number-
related expression of a goat bacterial artificial chromosome
alpha-lactalbumin gene in transgenic mice. *Biochem J* 339:
33–36.
- Strauss WM, Dausman J, Beard C, Johnson C, Lawrence JB and
Jaenisch R (1993) Germ line transmission of a yeast artificial
chromosome spanning the murine alpha 1(I) collagen locus.
Science 259: 1904–1907.
- Symula DJ, Frazer KA, Ueda Y, Deneffe P, Stevens ME, Wang ZE
et al. (1999) Functional screening of an asthma QTL in YAC
transgenic mice. *Nat Genet* 23: 241–244.
- Taboit-Dameron F, Malassagne B, Viglietta C, Puissant C, Leroux-
Coyau M, Chereau C et al. (1999) Association of the 5'HS4
sequence of the chicken beta-globin locus control region with
human EF1 alpha gene promoter induces ubiquitous and high ex-
pression of human CD55 and CD59 cDNAs in transgenic rabbits.
Transgenic Res 8: 223–235.
- Tanaka S, Yamamoto H, Takeuchi S and Takeuchi T (1990) Melan-
ization in albino mice transformed by introducing cloned mouse
tyrosinase gene. *Development* 108: 223–227.
- Tanimoto K, Liu Q, Bungert J and Engel JD (1999) Effects of altered
gene order or orientation of the locus control region on human
beta-globin gene expression in mice. *Nature* 398: 344–348.
- Umland T, Montoliu L and Schütz G (1997) The use of yeast
artificial chromosomes for transgenesis. In: Houdebine LM
(ed.) *Transgenic Animals, Generation and Use* (pp. 267–272)
Harwood Academic Publishers, The Netherlands.
- Vassaux G and Huxley C (1997) A dicistronic construct allows easy
detection of human CFTR expression from YAC DNA in human
cells. *Nucleic Acids Res* 25: 4167–4168.
- Vassilopoulos G, Navas PA, Skarpidi E, Peterson KR, Lowrey
CH, Papayannopoulou T et al. (1999) Correct function of the
locus control region may require passage through a nonerythroid
cellular environment. *Blood* 93: 703–712.
- Wallace H, Ansell R, Clark J and McWhir J (2000) Pre-selection of
integration sites imparts repeatable transgene expression. *Nucleic
Acids Res* 28: 1455–1464.
- Whitelaw CB, Archibald AL, Harris S, McClenaghan M, Simons
JP and Clark AJ (1991) Targeting expression to the mammary
gland: intronic sequences can enhance the efficiency of gene
expression in transgenic mice. *Transgenic Res* 1: 3–13.
- Wilson C, Bellen HJ and Gehring WJ (1990) Position effects on
eukaryotic gene expression. *Annu Rev Cell Biol* 6: 679–714.
- Wunderle VM, Critcher R, Hastie N, Goodfellow PN and Schedl
A (1998) Deletion of long-range regulatory elements upstream
of SOX9 causes campomelic dysplasia. *Proc Natl Acad Sci USA*
95: 10649–10654.
- Wutz A, Smrzka OW, Schweifer N, Schellander K, Wagner EF
and Barlow DP (1997) Imprinted expression of the Igf2r gene
depends on an intronic CpG island. *Nature* 389: 745–749.
- Yang XW, Model P and Heintz N (1997) Homologous recombina-
tion based modification in *Escherichia coli* and germline trans-
mission in transgenic mice of a bacterial artificial chromosome.
Nat Biotechnol 15: 859–865.
- Yang XW, Wynder C, Doughty ML and Heintz N (1999)
BAC-mediated gene-dosage analysis reveals a role for Zipr1
(Ru49/Zfp38) in progenitor cell proliferation in cerebellum and
skin. *Nat Genet* 22: 327–335.
- Yannoutsos N, Langford GA, Cozzi E, Lancaster R, Elsome K, Chen
P et al. (1995) Production of pigs transgenic for human regulators
of complement activation. *Transplant Proc* 27: 324–325.
- Yannoutsos N, Ijzermans JN, Harkes C, Bonthuis F, Zhou CY, White
D et al. (1996) A membrane cofactor protein transgenic mouse
model for the study of discordant xenograft rejection erratum.
Genes Cells 1: 409–419.
- Yu D, Ellis HM, Lee EC, Jenkins NA, Copeland NG and Court
DL (2000) An efficient recombination system for chromosome
engineering in *Escherichia coli*. *Proc Natl Acad Sci USA* 97:
5978–5983.
- Yu W, Misulovin Z, Suh H, Hardy RR, Jankovic M, Yannoutsos
N and Nussenzweig MC (1999) Coordinate regulation of RAG1
and RAG2 by cell type-specific DNA elements 5' of RAG2.
Science 285: 1080–1084.
- Zhang Y, Buchholz F, Muirers JP and Stewart AF (1998) A new
logic for DNA engineering using recombination in *Escherichia
coli*. *Nat Genet* 20: 123–128.
- Zhou Y, Lim KC, Onodera K, Takahashi S, Ohta J, Minegishi N
et al. (1998) Rescue of the embryonic lethal hematopoietic de-
fect reveals a critical role for GATA-2 in urogenital development.
EMBO J 17: 6689–6700.
- Zhu Y, Jong MC, Frazer KA, Gong E, Krauss RM, Cheng JF et al.
(2000) Genomic interval engineering of mice identifies a novel
modulator of triglyceride production. *Proc Natl Acad Sci USA*
97: 1137–1142.
- Zou X, Xian J, Davies NP, Popov AV and Bruggemann M (1996)
Dominant expression of a 1.3 Mb human Ig kappa locus repla-
cing mouse light chain production. *FASEB J* 10: 1227–1232.
- Zuelke KA (1998) Transgenic modification of cows milk for value-
added processing. *Reprod Fertil Dev* 10: 671–676.
- Zuo J, Treadaway J, Buckner TW and Fritsch B (1999) Visu-
alization of alpha9 acetylcholine receptor expression in hair
cells of transgenic mice containing a modified bacterial artificial
chromosome. *Proc Natl Acad Sci USA* 96: 14100–14105.
- Zweigerdt R, Braun T and Arnold HH (1997) Faithful expres-
sion of the Myf-5 gene during mouse myogenesis requires dis-
tant control regions: a transgene approach using yeast artificial
chromosomes. *Dev Biol* 192: 172–180.